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Colegio de Postgraduados

Characterization of weed flora in a cassava crop in Tabasco

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ABSTRACT

Objective: To evaluate the effect of the incorporation of *Crotalaria juncea* L. as green manure on the weed community in a cassava crop in Tabasco.

Design/methodology/approach: The study was carried out in the Experimental Field of Colegio de Postgraduados, Campus Tabasco (18° 01' N and 93° 03' W). The samplings were made on four dates: 1) at the planting of crotalaria (12/03/2018); 2) at flowering (31/01/2019); 3) 20 days after incorporation (22/02/2019); 4) at cassava harvest (25/04/2019), using metal squares 50×50 cm. Two planting densities, 50 and 80 cm (16,600 and 10,375 plants ha^{-1}), two doses of NPK fertilization (160-40-80 and 00-40-80+GM), and a control were tested. The name of the species, number of individuals and coverage (percentage) were recorded to calculate the richness (S), Shannon diversity (H') and uniformity (E) indices, and the importance value index (IVI).

Results: The weed community consisted of 32 species, 28 genera and 16 families, of which the best represented are: Convolvulaceae, Asteraceae, Cyperaceae and Poaceae. The most frequently recorded species are *Lindernia crustacea*, *Ludwigia octovalvis*, and *Ageratum houstonianum*. The diversity indices reflected a poor community, especially with GM treatments; diversity ranged from low to medium and uniformity from medium to high. The importance of the families was more related to the environmental conditions than to the treatments; Cyperaceae were more important in the rainy season and Asteraceae in the dry season. *L. crustacea* appeared throughout the cycle.

Limitations/implications: It is advisable to extend the investigation period.

Findings/conclusions: The weed flora consisted of 32 species; the Convolvulaceae family was the most diverse and *L. crustacea* was the most recorded species during the cycle. The diversity indices reflected a poor community. The importance of the families was related to environmental conditions, where Cyperaceae stood out in the rainy season and Asteraceae in the dry season.

Keywords: *Crotalaria juncea*, fertilization, green manure.

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INTRODUCTION

Cassava is one of the most important tropical crops in the world, and it serves as basic food and fodder for animals, in addition to having multiple uses in industry (Pinto-Zevallos *et al.*, 2016). In Mexico, it is cultivated in the states of Chiapas, Campeche, Yucatán, Oaxaca, Michoacán, Jalisco and Tabasco; the largest surface is reported in the latter, of 1,764 ha (SIAP, 2018). For their part, Rivera-Hernández *et al.* (2012) report that Tabasco has 476,617 hectares with high edaphoclimatic potential for cassava production, whose yield could reach 42.3 t ha^{-1} of fresh roots, which exceeds the averages: state (12.44), national (13.52) and global (10.50). The low yields are attributed mainly to the low fertility of the soil where it is cultivated and the excessive use of chemical products such as fertilizers, pesticides and herbicides (López-López *et al.*, 2018).

On the other hand, competition with weeds decreases the yield of crops, generally in 10%; however, if no control is exerted, it could decrease so much that it could cause the total loss due to competition over light, water and nutrients. In cassava, weed infestation since 15 days after the budbreak of cuttings causes an important decrease in the yield, so it is advisable to control weeds until the plantation has dense foliage, which can take about four months. For weed control, there are different options: cultural, mechanic, chemical or a combination of these. Chemical control is based on the use of herbicides, but its excessive use causes economic losses, environmental damage, and harm to health (Rubiano Rodríguez and Cordero-Cordero, 2019).

This makes sustainable alternatives for the management of this crop necessary, where technologies such as the use of green fertilizers (GF), among others, could allow maintaining or improving their yield and controlling weeds, thus preventing or minimizing the use of agrichemicals (Prager *et al.*, 2012). Legumes used as GF have the ability to improve soil fertility and to fix atmospheric nitrogen. In this context, *Crotalaria juncea* L. germinates and develops quickly, it has a dense growth habit so there can be an impact on weed control, and it reduces the population of nematodes in the soil; in addition, the species fixes atmospheric nitrogen and produces abundant organic matter which contributes N to the system (Skinner *et al.*, 2012). The objective of this study was to evaluate the effect of the cultivation and incorporation of *Crotalaria juncea* L. as green fertilizer, on the weed community in a cassava crop.

MATERIALS AND METHODS

The research was carried out in the Experimental Field of Colegio de Postgraduados, Campus Tabasco, located on Km 21 of the Federal Highway 180 Cárdenas-Coatzacoalcos, in Tabasco, Mexico (18° 01’ N - 93° 03’ W) from September 2018 to April 2019. The climate is tropical warm-humid with abundant summer rains (Am(g)w”) with annual precipitation of 2,342 mm; the mean annual temperature is 26 °C and the soil is a clayey eutric Cambisol (CMeu) (Palma-López *et al.*, 2007).

The weed samples were made using metal squares of 50×50 cm (Mostacedo and Fredericksen, 2000) in four stages: 1) at the time of sowing *Crotalaria juncea* L. (03/12/2018); 2) at flowering (31/01/2019), 3) 20 days after incorporating the GF (22/02/2019); and 4) at the time of harvesting cassava (25/04/2019). Two sowing densities were studied, two fertilization treatments plus a control: 1) C: Control; 2) GF-50: with GF+PK (00-40-80) and 50 cm distance between plants (16,600 plants ha^{-1}); 3) GF-80: with GF+PK (00-40-80) and distance of 80 cm (10,375 plants ha^{-1}); 4) D-50: with doses of NPK fertilizer (160-40-80) and distance of 50; 5) D-80: with NPK fertilizer (160-40-80) and distance of 80 cm.

For each weed species sampled, the name (common and/or scientific) was recorded, the number of individuals, and the percentage of coverage. All the plants that were inside the squares were collected, to be taken to the CSAT Herbarium, where their taxonomic identity was verified through the use of specialized bibliography, a stereoscopic microscope, and verification of botanized specimens. The data were systematized and analyzed in Excel 2007 to calculate the following indices: richness (S), Shannon diversity (H'), uniformity (E) (Magurran, 1988); and the importance value index (IVI), including the absolute and relative values of its components: Density (De, rDe), Frequency (Fr, rFr) and Dominance (Do, rDo) (Concenço *et al.*, 2016).

RESULTS AND DISCUSSION

The floristic list of weeds in the cassava crop included 32 species that were placed in 28 genera and 16 botanical families (Table 1); 26 species are dicotyledonous (81.2%) and similar values were reported by García-Jiménez (2015), Naranjo-Landero (2020) and Obrador-Olán *et al.* (2019) in sugarcane in the same region. The best represented families were: Convolvulaceae with 5 species, and Asteraceae, Cyperaceae and Poaceae with 3 each. It is evident that no previous study had reported Convolvulaceae as the most diverse family in the region; the species sampled in the area have crawling or climbing habit, or both, which eases their development and distribution (Carranza, 2008a, 2008b). Among the species that are most frequently recorded in the cassava crop, there are: *L. crustacea*, which was present in all the samples and treatments, except in C in January and GF-50 in April; it is a typical plant of flooded sites present with the northern winds season (Trópicos, 2019); these conditions also eased the presence of clavillo (*L. octovalvis*), which was practically not found in the sample from the driest month, pincel (*A. houstonianum*) and navajuela (*S. setuloso-ciliata*).

The behavior of richness (S), diversity (H') and uniformity (E) of the weed community in the cassava crop is presented in Figure 1. The number of species (S) presented the lowest values in the first sample, with it being generally higher where complete fertilization was applied, and lower in C; in the last sample all the values decreased, except in C. The diversity (H') varied from low (<1.5) to medium (1.5-3.5), except in C, where it was low during the entire cycle; the highest values were observed where the distance of planting was greater; due to the availability of more surface and light, the weeds developed more vegetative growth (Blanco-Valdez, 2016). In the four treatments, H' decreased at harvesting of the cassava because of a greater development of the crop and the consequent increase of shade, which eventually affected weed growth negatively (Aristizábal *et al.*, 2007).

For its part, the Uniformity (E) tended to increase its value from medium to high in the four treatments, decreasing in the last sample, which highlights its inverse relationship with species richness (Perdomo *et al.*, 2004); in general, the lowest values were seen in C.

Table 2 shows the values of the IVI from the three main weeds by sample and treatment. The species that stood out in the first sample, when the crotalaria was planted, was navajuela, which had the highest IVI in C, where it far exceeded the other species, and in D-50, where it was surpassed in density by *F. dichotoma* which occupied the first

Table 1. Floristic listing of weed species present in a cassava crop, under two densities of plantation and two doses of fertilization, in Tabasco, Mexico.

	Family		**Species/sampling * Treatments**	**03/Dec/2018**					**31/Jan/2019**					**22/Feb/2019**					**25/Apr/2019**				
				1	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
			Dicotyledons																				
1	Asteraceae	1	*Acmella repens* (Walter) Rich.						x					x			x	x	x	x	x	x	x
		2	*Ageratum houstonianum* P. Mill.		x	x	x	x	x	x	x	x	x		x	x	x	x					
		3	*Melanthera nivea* (L.) Small					x										x					
2	Capparaceae	4	*Cleome viscosa* L.	x		x		x							x								
3	Convolvulaceae	5	*Ipomoea purpurea* (L.) Roth		x	x	x												x	x	x		x
		6	*Ipomoea trífida* (L.) Lam												x								
		7	*Ipomoea triloba* L.													x		x		x		x	
		8	*Jacquemontia thamnifolia* (L.) Griseb									x			x	x							
		9	*Camonea umbellata* (L.) Simões & Staples								x		x				x	x					
4	Euphorbiaceae	10	*Caperonia palustris* (L.) A. St.-Hill																		x	x	
		11	*Euphorbia hirta* L.			x																	
5	Fabaceae	12	*Crotalaria juncea* L.		x	x				x													
		13	*Mimosa pudica* L.					x				x			x		x		x	x	x	x	x
6	Lamiaceae	14	*Hyptis brevipes* Poit															x					
7	Linderniaceae	15	*Lindernia crustacea* (L.) F.Muell.	x	x	x	x	x		x	x	x	x	x	x	x	x	x	x	x		x	x
		16	*Lindernia dubia* (L.) Pennel.								x		x				x	x					x
8	Loganiaceae	17	*Spigelia anthelmia* L.					x															
9	Lythraceae	18	*Ammannia coccinea* Rottb.,											x					x				
		19	*Cuphea carthagenensis* J.F.Macbr.						x					x	x	x							x
10	Malvaceae	20	*Corchorus orinocensis* Kunth										x										
		21	*Melochia pyramidata* L.				x									x							
11	Molluginaceae	22	*Mollugo verticillata* L.								x	x	x		x	x	x	x					
12	Onagraceae	23	*Ludwigia octovalvis* (Jacq.) P.H. Raven		x	x	x	x		x	x	x	x		x	x	x	x			x		
13	Phyllanthaceae	24	*Phyllanthus niruri* L.			x	x			x	x	x	x					x				x	
		25	*Phyllanthus urinaria* L.			x						x			x	x	x			x	x	x	
14	Scophulariaceae	26	*Bacopa procumbens* (Mill.) Greenm.															x					
			Monocotyledons																				
15	Cyperaceae	27	*Cyperus rotundus* L.	x	x	x	x	x									x		x				x
		28	*Fimbristylis dichotoma* (L.) Vahl	x		x	x	x					x										
		29	*Scleria setuloso-ciliata* Boeckeler	x	x	x	x	x	x	x	x		x		x								
16	Poaceae	30	*Echinochloa colona* (L.) Link													x	x	x				x	
		31	*Paspalum fasciculatum* Willd. ex Flüggé										x										
		32	*Paspalum notatum* Flüggé						x	x		x	x										

* Treatments: 1) Control; 2) DB-NPK; 3) DB-PK-AV; 4) DA-NPK; 5) DA-PK-AV.

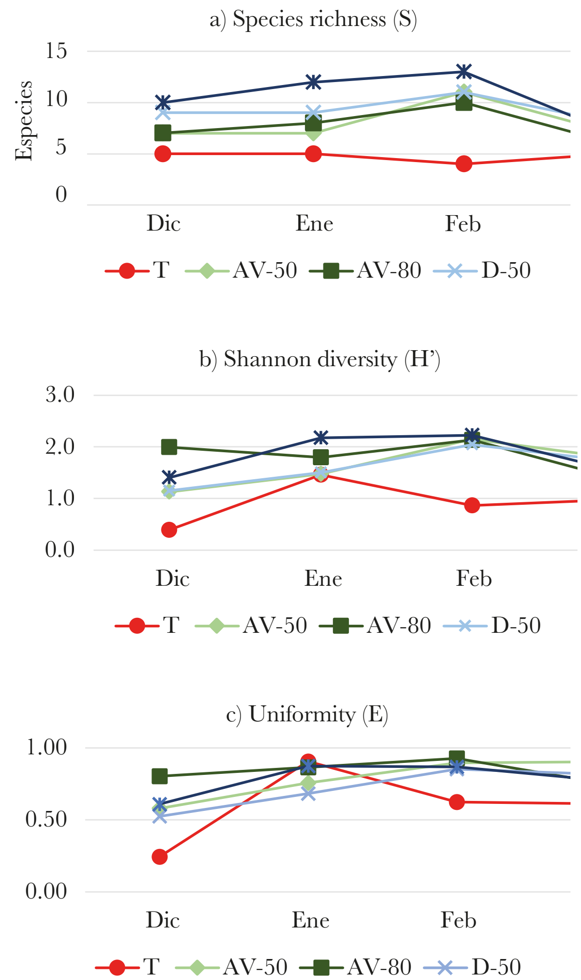

Figure 1. Behavior of: a) richness (S), b) diversity (H') and c) uniformity (E) of weeds in the cassava crop with two fertilization treatments and two planting distances: control (C), GF and 50 cm (GF-50), GF and 80 cm (GF-80), fertilization NPK and 50 cm (D-50), and fertilization NPK and 80 cm (AV-80).

place in GF -80; these and coquillo (*C. rotundus*), which stood out in C, GF-50 and D-80, belong to the Cyperaceae family. The species *L. crustacea* was the main one in GF-50 and D-80. Coquillo is an introduced perennial plant, considered as the most important weed in the tropics, since it has been found in more countries, regions and localities of the world (Torres and Ortiz, 2022). All the plants mentioned before tending to develop well in flooded, swamped soils, stream banks, small lagoons, canals, and fields flooded during the rainy season in the study area (Vibrans, 2009; Rzedowski and Rzedowski, 2008). Campanita (*I. purpurea*), which occupied the second place in GF-80 and third in D-50, has been recorded as weed in at least 25 crops where dense populations that climb over the crops can form, making their harvest difficult (Villaseñor and Espinosa, 1998). Finally, *C. viscosa*, which is characterized by a sticky pubescence and unpleasant smell, was in the second position in C, is native to Asia, although it currently presents a pantropical distribution (Guzmán-Vázquez and Quintanar-Castillo, 2017).

Table 2. Importance value index (IVI) and its components: Relative Density (DeR), Relative Frequency (FR) and Relative Dominance (DoR) of the three main weeds in a cassava crop under two planting densities and two fertilization doses in Tabasco.

Sampling 1 (03-12-2018)					Sampling 2 (31-01-2019)				
Name	**DeR**	**FR**	**DoR**	**IVI**	**Name**	**DeR**	**FR**	**DoR**	**IVI**
Control					**Control**				
S. setuloso-ciliata	91.7	44.4	85.8	221.9	*A. repens*	38.6	22.2	23.5	84.3
C. viscosa	3.6	22.2	7.5	33.3	*A. houstonianum*	22.8	22.2	34.1	79.1
C. rotundus	2.4	11.1	4.0	17.5	*S. setuloso-ciliata*	21.1	22.2	15.3	58.6
Green manure-50 cm					**Green manure-50 cm**				
L. crustacea	54.9	37.5	27.9	120.3	*L. crustacea*	48.0	27.9	29.3	105.2
S. setuloso-ciliata	30.9	25.0	43.9	99.7	*C. juncea*	25.2	24.8	23.0	73.0
C. rotundus	3.1	12.5	8.6	24.2	*A. houstonianum*	8.0	13.9	13.2	35.2
Green manure-80 cm					**Green manure-80 cm**				
F. dichotoma	37.5	25.8	37.1	100.4	*L. crustacea*	40.9	20.2	26.5	87.5
I. purpurea	8.1	7.1	28.8	44.1	*L. octavalvis*	13.2	13.0	19.7	45.9
L. crustacea	18.9	14.3	6.4	39.6	*L. dubia*	10.5	16.0	13.9	40.3
Dose-50 cm					**Dose-50 cm**				
S. setuloso-ciliata	25.1	24.2	24.4	73.7	*L. crustacea*	46.6	30.0	26.1	102.7
F. dichotoma	33.1	16.5	20.8	70.4	*A. houstonianum*	20.8	15.8	26.4	63.1
I. purpurea	10.1	20.4	33.1	63.6	*L. octavalvis*	17.8	25.8	18.3	61.9
Dose-80 cm					**Dose-80 cm**				
L. crustacea	50.1	25.0	29.8	104.9	*A. houstonianum*	16.6	17.7	23.0	57.3
C. rotundus	21.8	21.9	25.0	68.7	*C. umbellata*	9.2	15.3	17.1	41.6
S. setuloso-ciliata	10.5	15.6	21.9	48.1	*L. octavalvis*	11.3	17.7	10.8	39.8
Sampling 3 (22-02-2019)					**Sampling 4 (02-04-2019)**				
Control					**Control**				
A. repens	54.7	33.3	57.6	145.6	*A. repens*	67.8	37.5	48.5	153.8
A. coccinea	40.7	33.3	35.9	110.0	*L. crustacea*	10.3	25.0	24.5	59.9
L. crustacea	3.5	22.2	4.0	29.7	*I. purpurea*	16.1	12.5	20.2	48.8
Green manure-50 cm					**Green manure-50 cm**				
A. houstonianum	16.4	10.3	22.7	49.4	*M. pudica*	13.3	13.8	32.2	59.3
I. trifida	13.0	15.5	17.7	46.2	*L. crustacea*	23.9	15.4	18.5	57.8
L. crustacea	19.7	15.3	9.5	44.5	*I. purpurea*	14.3	21.5	16.8	52.6
Green manure-80 cm					**Green manure-80 cm**				
P. urinaria	14.3	18.2	20.2	52.6	*M. pudica*	27.8	28.6	36.1	92.4
A. houstonianum	20.8	13.6	14.5	49.0	*A. repens*	33.9	20.0	31.1	85.0
J. thamnifolia	11.4	18.2	16.2	45.7	*C. palustris*	23.1	24.3	17.9	65.3
Dose-50 cm					**Dose-50 cm**				
C. umbellata	12.2	15.9	21.2	49.3	*A. repens*	38.0	27.4	36.8	102.2
A. repens	22.2	4.2	20.3	46.7	*M. pudica*	28.0	31.0	30.8	89.8
L. dubia	19.8	11.8	13.6	45.2	*I. triloba*	10.0	10.7	14.2	34.9
Dose-80 cm					**Dose-80 cm**				
A. houstonianum	19.1	14.6	45.6	79.3	*A. repens*	34.1	22.2	40.3	96.6
A. repens	15.9	12.5	8.3	36.7	*M. pudica*	34.6	12.5	29.5	76.6
M. verticillata	12.7	14.6	7.3	34.6	*I. purpurea*	11.3	27.8	16.9	56.0

In the sample from January, flowering from the GF, *L. crustacea* was the most important species in the treatments GF-50, GF-80 and D-50. Pincel, an Asteraceae that had the highest IVI in D-80, the second in C and D-50, and the third in GF-50, has a fleeting annual habit that is associated with its great plasticity in the growth form, development speed, great seed production, and efficient use of carbon (Singh *et al.*, 2011). The presence of clavillo (*L. octovalvis*), in the second place in GF-80 and third in D-50 and D-80, tends to be associated to conditions of high moisture in the soil (Vibrans, 2009). Botón de oro (*A. repens*), the one with highest IVI in C, is also an Asteraceae, family of invaders because they are very efficient in the use of resources and due to their plasticity in terms of development and seed production (García-López, 1990). Bejuco (*C. umbellata*), which occupied the second place in D-80, is a Convulvulaceae that due to its climbing habit tends to cause problems in the harvest of several tropical crops; crotalaria occupied the second place in GF-50, while navajuela and *L. dubia*, species that is quite close to *L. crustacea*, had the third place in C and GF-80, respectively.

In the sample from February 22, a month after the incorporation of the GF, a better development of pincel was noted; it had the highest IVI in GF-50 and D-80, and the second in GF-80; and botón de oro, which appeared again with the highest IVI in C and second place in D-50 and D-80, although in D-50 it exceeded bejuco in density. The species of *Lindernia* appeared in third place: *L. crustacea* in C and GF-50, which in the latter had the highest value in density, while in D-50, *L. dubia* exceeded botón de oro in frequency. *P. urinaria* was the most important in GF-80, it is originally from Asia, and high contents of N have been found in it, particularly in buds of plants under shade conditions, with a positive correlation observed with soils with good content of that element (Dogra *et al.*, 1978). In the second site in the control plot, *A. coccinea* was found, which has been reported as a very aggressive weed in rice crops in many countries and apparently is dispersed by mixing with the crop's seed (Graham, 1991). *I. trífida* occupied the second place in GF-50, although it surpassed pincel in frequency, which was also surpassed in frequency and dominance by *J. tamnifolia* in GF-80, where it occupied the third place; the advantage of the two Convulvulaceae is that they have crawling and voluble growth habit, which gives them the capacity of climbing over other plants and extending over the ground, so that one or a few individuals can cover important surfaces (Carranza, 2008a, 2008b). Finally, although *M. verticillata* had third place in D-80 and exceeded botón de oro in frequency, it is a species of undefined origin, although with a wide distribution area and has been reported as weed in corn, sugarcane, rice, bean and tomato (Ocampo-Acosta, 2002; Villaseñor and Espinosa, 1998).

For the month of April, just before the cassava harvest, botón de oro was the most important species in C, D-50 and D-80, and the second one in GF-80. By this date, the presence of dormilona (*M. pudica*) also stood out, which presented the highest IVI in the two plots with GF, and occupied the second position in the two fertilized; it is a dangerous weed because its stems are protected by spines of up to 7 mm and a wide base, which tends to form dense populations reducing the impact of light on the ground level, so many plants do not manage to persist and form monospecific shrubs (Witt *et al.*, 2020). *L. crustacea* was found in the second place in the C and GF-50 plots, campanita was found occupying the third place in the same ones and in D-80, *I. triloba* in D-50, and *C. palustris* in GF-80, which is reported as annual and perennial. Koger *et al*. (2004) point out that a plant produces ±900 seeds with 90% viability; the seeds germinate at between 30 and 40 °C but not under flooding, although the seedlings can survive to it for several weeks.

In general, the presence or importance of the species with the highest IVI do not seem to be related to the type of fertilization or the density of cassava plants, but rather with the environmental conditions associated to the region's climate. In this sense, Cyperaceae stood out in the first sampling: navajuela, coquillo and *F. dichotoma*, when the soil remained mostly flooded. Of the Asteraceae, pincel was very important in the January and February samples, and although it appears in January, botón de oro stood out in February and April, as the soil was becoming drier; the fleeting annual habit of this group is associated with its huge plasticity when it comes to its growth form, flowering speed, and high seed production, which is related to the efficient use of carbon and the development of competitive strategies such as the increase in biomass and relative growth rate (García-López, 1990). Dormilona was notable in February and April, its presence also coinciding with the decrease in soil moisture. Instead, the importance of clavillo was concentrated in the month of January, and *L. crustacea* stood out during the entire cycle.

CONCLUSIONS

The community of weeds in this study consisted of 32 species, 28 genera and 16 families, and the most represented were Convolvulaceae, Asteraceae, Cyperaceae and Poaceae. The most frequent species in the samples were *Lindernia crustacea*, *Ludwigia octovalvis* (clavillo) and *Ageratum houstonianum* (pincel). The diversity indices reflect a poor community. The lowest richness was found in the Control during the entire cycle, increasing in the treatments with lower cassava density and incorporation of Green Fertilizer. The treatments with Green Fertilizer were poorer in weeds than the treatments with fertilizers. The diversity varied from low to medium, being highest in the treatments with lowest density of plantation; in the Control it was low during the entire cultivation cycle. The uniformity varied from medium to high. The abundance of weeds was more related with environmental conditions than with the density of cultivation and fertilization. The Cyperaceae were more important in the first two samples, which coincide with the rainy season, while the Asteraceae and *Mimosa púdica* (dormilona) stood out from January to April, when the soil was drier. *Lindernia crustacea* was present during the entire cultivation cycle.

REFERENCES

Aristizábal J. T. Sánchez y D. Mejía-Loríо. 2007. Guía técnica para producción y análisis de almidón de yuca. Boletín de Servicios Agrícolas de la FAO 163. Organización de las Naciones Unidas para la Agricultura y la Alimentación. Roma.

Blanco-Valdez, Y. 2016. El rol de las arvenses como componente en la biodiversidad de los agroecosistemas. *Cultivos tropicales 37*(4): 34-56

Carranza E. 2008a. Diversidad del género *Ipomoea* L. (Convolvulaceae) en el estado de Michoacán, México. Flora del Bajío y de Regiones Adyacentes. Fascículo complementario 23. Instituto de Ecología, Centro Regional del Bajío. Pátzcuaro, Michoacán, México. 122 p.

Carranza E. 2008b. Convolvulaceae II. Flora del Bajío y de Regiones Adyacentes. Fascículo 155. Instituto de Ecología, Centro Regional del Bajío. Pátzcuaro, Michoacán, México. 109 p.

Concenço G., R.A. Leme Filho J.R.A., C.J. Silva, C., R.F. Marques, L.B.X. Silva, I.V.T. Correia. 2016. Weed occurrence in sugarcane as function of variety and ground straw management. *Planta Daninha 34*(2): 219-228 p.

Dogra J.V.V., S. K. Sinha P., y S.G. Abbas. 1978. Variation in the total nitrogen content in *Phyllanthus urinaria* Linn. as influenced by soil nitrogen. *Comparative Physiology and Ecology 3*(2): 79-80 p.

García-Jiménez P. 2015. Flora asociada al cultivo de caña de azúcar (*Saccharum* sp.) en el municipio de Cárdenas, Tabasco. Tesis. Tabasco, México: Instituto Tecnológico de la Zona Olmeca.

García-López E. 1990. Las compuestas silvestres del Valle de Morelia, Michoacán México. Tesis de licenciatura. Escuela de Biología, Universidad Michoacana de San Nicolás de Hidalgo, Morelia, Michoacán. 1-247 p.

Graham S. A. 1991. Lythraceae. Flora de Veracruz. Fascículo 66. Instituto de Ecología, A.C. Xalapa, Veracruz, México. 9-12 p.

Guzmán-Vázquez I., y A. Quintanar-Castillo. 2017. Cleomaceae. Flora de Guerrero. Fascículo 74. Universidad Nacional Autónoma de México, Facultad de Ciencias. México. 1-44 p.

Koger H.C., K. Reddy N., and D. Poston H. 2004. Factors affecting seed germination, seedling emergence, and survival of texas weed (*Caperonia palustris*). *Weed Science* 52: 989-995 p.

Magurran A.E. 1988. Diversidad Ecológica y su medición. Ediciones Vedra, Barcelona, España. 198 p.

López-López R., M.A. Ramírez-Guillermo y J. Martínez-Herrera, J. Cámara-Córdova, A. Durán-Prado. 2018. Respuesta de la yuca para raíz *Manihot esculenta* Krantz a la fertilización con NPK en un Acrisol húmico en Tabasco, México. In: Investigaciones Científicas y Agrotecnológicas para la Seguridad Alimentaria. Universidad Juárez Autónoma de Tabasco. p. 114-123.

Mostacedo B., y S. Fredericksen T. 2000 Manual de métodos básicos de muestreo y análisis en ecología vegetal. Santa Cruz, Bolivia. 21-87 p.

Naranjo-Landero S., J.J. Obrador-Olán, E. García-López, A. Valdez-Balero, V.I. Domínguez-Rodríguez. 2020. Arvenses en un suelo cultivado con caña de azúcar con fertilización mineral y abono verde. *Polibotanica* 50: 119-135.

Obrador-Olán J.J., E. García-López, L.E. Almeyda-Santos, M. Castelán-Estrada y E. Carrillo-Ávila. 2019. Weeds in a sugar cane soil cultivated with *Crotalaria juncea*. *Planta Daninha* 37:1-10.

Ocampo-Acosta G. 2002. Molluginaceae. Flora del Bajío y de Regiones Adyacentes, Fascículo 101. Instituto de Ecología, A.C. Centro Regional del Bajío. Pátzcuaro, Michoacán. 1-11 p.

Palma-López D.J., J. Cisneros D., E. Moreno C. y J.A. Rincón-Ramírez. 2007. Suelos de Tabasco: su uso y manejo sustentable. Colegio de Postgraduados-ISPROTAB-Fundación Produce Tabasco. Villahermosa, Tabasco, México. 195 p.

Perdomo R.F., H. Vibrans L., A. Romero M., J.A. Domínguez V. y J.L. Medina P. 2004. SHE, una herramienta para estudiar la diversidad de maleza. *Revista Fitotecnia Mexicana 27*(1): 57-60p.

Pinto-Zevallos D.M., M. Pareja and B.G Ambrogi. 2016. Current knowledge and future research perspectives on cassava (*Manihot esculenta* Crantz) chemical defenses: an agroecological view. *Phytochemistry* 130: 10–21.

Prager-Mósquera M. O.E. Sanclemente-Reyes, M. Sánchez-Prager, J. Miller-Gallego, D.I. Ángel-Sánchez. 2012. Abonos verdes: tecnología para el manejo agroecológico de los cultivos. *Agroecología* 7: 53-62.

Rivera-Hernández B., L.A. Aceves-Navarro, F.J Juárez-López, D.J. Palma-López, R. González-Mancillas y V. González-Jiménez. 2012. Zonificación agroecológica y estimación del rendimiento potencial del cultivo de la yuca (*Manihot esculenta* Crantz) en el estado de Tabasco, México. *Avances en Investigación Agropecuaria 16*(1): 29-47p.

Rubiano-Rodríguez J.A. y C.C. Cordero-Cordero. 2019. Épocas críticas de competencia de arvenses en cultivo de yuca en el Caribe seco colombiano. *Temas Agrarios 24*(2): 108-118.

Rzedowski J. y G. Calderon R. 2008. Compositae. Tribu Heliantheae I: géneros Acmella-Jefea. Flora del Bajío y de Regiones Adyacentes. Fascículo 157. Instituto de Ecología, Centro Regional del Bajío. Pátzcuaro, Michoacán, México. 12-20 p.

SIAP (Sistema de Información Agroalimentaria y Pesquera). 2018. Cierre de producción agrícola por cultivo. Anuario Estadístico de la Producción Agrícola.2018. En: https://nube.siap.gob.mx/cierreagricola/. Consultado el 15/11/2019.

Singh, V. H. Singh, G.P. Sharma and A.S. Raghubanshi. 2011. Eco-physiological performance of two invasive weed congeners (*Ageratum conyzoides* L. and *Ageratum houstonianum* Mill.) in the Indo-Gangetic plains of India. *Environ Monit Assess* 178: 415–422.

Skinner E.M., J.C. Díaz-Pérez S.C. Phatak H.H. Schomberg y W. Vencill. 2012. Allelopathic effects of sunnhemp (*Crotalaria juncea* L.) on germination of vegetables and weeds. *Hortscience 47*(1): 138-142

Torres S. y A. Ortiz. 2022. Estudio fitosociológico y evaluación del banco de malezas del suelo en tres fincas maiceras del Estado Portuguesa, Venezuela. *Bioagro 34*(1): 27-38.

Tropicos 2019. Linderniaceae: *Lindernia crustacea* (L.) F.Muell. Tropicos.org. Missouri Botanical Garden. En: http://www.tropicos.org/Name/29201037. Consultado el 30 de Oct de 2019.

Vibrans H. 2009. Malezas de México. http://www.conabio.gob.mx/malezasdemexico/2inicio/paginas/lista-plantas.htm. Consultado el 15/11/2019.

Villaseñor-Ríos J.L. y F.J. Espinosa-García. 1998. Catálogo de malezas de México. Universidad Nacional Autónoma de México. Consejo Nacional Consultivo Fitosanitario. Fondo de Cultura Económica. México. 448 p.

Witt A.B.R., K.S. Floyd, W. Nunda, T. Beale, G. Shanungu and D.J. Kriticos. 2020. *Mimosa pigra* in eastern and southern Africa: Distribution and socio-ecological impacts. *Austral Ecology* 45: 788–799.

Colegio de Postgraduados

Origin, history, and current situation of donkeys and mules in Mexico

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ABSTRACT

Objective: To review the economic and social importance of donkeys and mules and their current situation in Mexico.

Design/Methodology/Approach: Donkeys and mules have contributed to human development for thousands of years, mainly as pack animals, helping in the development of trade and the transportation of people. These strong, hardy, and long-lived animals can easily adapt to different environmental conditions, are resistant to diseases, and tolerate heavier loads than horses.

Study Limitations/Implications: These animals are mainly found in developing countries, where they are used for transportation, as beasts of burden, and for agricultural work. Unfortunately, their welfare is threatened as their basic needs (*e.g.*, feeding, health, and housing) are not met, despite their undeniable usefulness, especially in rural conditions.

Findings/Conclusions: Donkeys and mules have played a major role and made an undeniable contribution to human civilizations. Currently, they mainly support people in developing countries, helping them carry out their daily chores and earn income.

Keywords: Donkeys, mules, economic and social importance.

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INTRODUCTION

Donkeys and mules are species that have been an important part of people's lives for thousands of years (Kimura *et al.*, 2013). They have great social, economic, and cultural value in the poorest communities worldwide, because they help to improve people's quality of life (Norris, 2021). These animals are of great help to the elderly, women, and children (Davis, 2019). They have a stoic nature and can survive in difficult regions, because their digestive system allows them to optimize poor quality fibrous feeds. Consequently, they are ideally suited for working in areas with extreme climates (Smith, 2016), especially donkeys,

due to their smaller size, ease of handling, and ability to work in small spaces (Queiroz *et al.*, 2021). However, they are not considered am economically transcendental species, unlike other species which generate products for direct consumption (Norris *et al.*, 2021), including meat, milk, and offspring.

The global donkey and mule population is estimated at around 50 million (FAO, 2021). Currently the demand of both donkeys and mules for recreational purposes and agricultural activities is increasing, while donkeys are used for the production of mules, milk, and skin. Both species are appreciated for their great intelligence, security, and work sturdiness (Smith, 2016).

The origin of donkeys and their participation in the development of humanity

The domestic donkey is a direct descendant of the African wild donkey and has two maternal origins, the Nubian donkey (*Equus africanus africanus*) and the Somali donkey (*Equus asinus somaliensis*) (Seyiti and Kelimu, 2021). There is still controversy regarding the place or places where domestication occurred, given that the origins and dates remain uncertain (Rosenbom *et al.*, 2015). However, evidence in murals and fossil remains indicate that donkeys were first domesticated in northern Egypt, where they were used to produce meat and milk (Mayers, 2017). Nevertheless, further work is still necessary on the DNA analysis of the remains found in these places, before a precise place and date of their domestication can be established (Mitchell, 2018).

Seyiti and Kelimu (2021) have theorized that the donkey was domesticated approximately 5,000 years ago by African cattle herders. As a domestic animal, it played an important role in the development and expansion of human civilization, especially in trade (Camillo *et al.*, 2018; McLean *et al.*, 2019). The resulting economic growth (Ali *et al.*, 2015) has influenced the culture of these societies (Seyiti and Kelimu, 2021). Its domestication caused a notable change in the transportation systems of both Africa and Asia, which increased the mobilization of people and goods. They also influenced the organization of the first cities and societies, mainly those that consisted of cattle herders (Rosenbom *et al.*, 2015). Over time and, as a result of the arrival of mechanization, there has been a considerable decrease in the donkey population worldwide (Kugler *et al.*, 2008). Nevertheless, they have never stopped playing an important role in developing countries, where they participate in traction, burden-carrying, and transporting activities, therefore contributing to the subsistence of millions of people (Camillo *et al.*, 2018).

Donkeys are known to be docile animals, regardless of their size: they range from miniature donkeys ($<$0.90 m) to mammoths and Andalusians, which can reach a height of 1.60 m (Geor, 2013; Smith, 2016). Nowadays, donkeys come in different sizes and have diverse coat textures and colors, with gray, brown, black, roan, white, and their combinations being the most common shades (Mayers, 2017).

Donkey breeds

The diversity of breeds is the result of the various crosses that took place over 2,000 years ago in their journeys along the trade routes that made up the Silk Road (Mayers, 2017). According to FAO (2021) and Norris *et al.* (2021), approximately 163 breeds of donkeys are distributed throughout the world. China has the greatest diversity, with a total of 21 breeds, while Europe is estimated to be home to 17 breeds (The Donkey Sanctuary, 2019), most of which are found in Italy, France, and Spain. Five other breeds are found in Ethiopia, India, Iran, Mali, and Sudan. On the Americas, Venezuela is the country with the greatest number of breeds (6), followed by Brazil and Bolivia (3 each) and Cuba and El Salvador (2 each).

Although the FAO (2021) only reports one native breed in Mexico, there is already a recently created 100% Mexican breed which consequently does not appear in the organization's statistics: the Mixtec donkey. This breed was created by the Asociación Nacional de Criadores de Burros y Mulas, in collaboration with the Universidad de Chihuahua, and was officially presented in 2021 (Panorama Agropecuario, 2021).

The donkey in the Americas

The arrival of the donkey to the American continent is attributed to Christopher Columbus, who asked the kings of Spain for permission to take donkeys during his second voyage (1494), in which he reached modern day Puerto Rico and Cuba. The conquistadores who followed Colón were responsible for taking donkeys to South and Central America and soon the donkey population in the region increased (Smith, 2016). In 1531, 14 donkeys were aboard the ships that sailed from Sevilla to Nueva España, in order to begin mule breeding, which soon became a large-scale activity (Australia, 2019).

Donkeys in Mexico

The first donkeys in Mexico were brought from Arizona by Juan de Oñate in 1598, with the purpose of using them in exploration activities and as draught animals in mining. Once the mining peak ended, donkeys were despised and abandoned in the deserts and wild donkeys are still found in the western United States (Smith, 2016). However, since 1991, the number of donkeys began to decrease drastically and they have even been declared endangered (National Geographic, 2022).

Nevertheless, several non-governmental organizations in Mexico are working on the rescue, protection, and production of donkeys. Burrolandia, located in the municipality of Otumba, State of Mexico, is home to donkeys rescued from abuse. For its part, Granja la Esperanza, a sanctuary located in the municipality of Españita, Tlaxcala, focuses on the production of dairy donkeys, with the ultimate objective of creating a dairy breed and increasing the donkey population. Another example is the Asociación Nacional de Criadores de Mulas y Burros, which has taken on the task of promoting the production of both mules and donkeys (México Desconocido, 2020; González, 2021).

Donkeys are mostly found in poorly developed rural areas and are considered a means of livelihood for many families (Alvarado-Arellano *et al.*, 2011). They are used as beasts of burden and for transportation and draught agricultural activities and they can even be easily handled by children (Tadich *et al.*, 2016). Most donkeys in the country lack good management and they received poor medical care in comparison with horses. Donkey livestock is found throughout the national territory and the largest population

Table 1. Major donkey breeds in the world nowadays.

Raza	Descripción
Abisinio	Conocido también como burro etíope, animal de carga en comunidades pobres de Etiopía.
Bourbonnais	Raza originaria de Francia. Los machos alcanzan una altura máxima de 1.35 m y las hembras 1.28 m, el color representativo es marrón chocolate, con capas grisáceas en la parte del hocico y vientre, las extremidades pueden presentar rayas de cebra.
Cotentín	Raza de la región de Normandía en el noreste de Francia.
Andaluz ó cordobés	Nativo de la provincia de Córdoba en Andalucía, España. Considerada la raza más antigua con 3000 años de existencia. Una altura máxima de 1.6 m, el color predominante es el gris, en ocasiones blanco, de pelo muy fino y suave. Es un animal fuerte y robusto, muy dócil .
Normando	Nativo del noreste de Francia, la raza más pequeña de Francia, una alzada máxima de 1.25 m, utilizada en deportes, turismo y recreación. .
Marroquí o *Raçca Asenca Balear*	Nativo de la costa este de España, con mayor presencia en Mallorca y Menorca, en 2007 fue declarada en peligro de extinción.
Burro de las Encartaciones	Nativo de España, hoy se encuentra en peligro de extinción. Única raza pequeña en España con una altura máxima de 1.2 m y un peso aproximado de 200 kg, capa de color negro, vientre, hocico y bordes de los ojos de color pálido. Su carne era considerada un manjar por lo que eran sacrificados a los tres meses de edad.
Burro de Poitou o Poitevin	Raza originaria de Francia, es de las razas más grandes por lo que se utiliza en la producción de mulares de trabajo.
Burro corso	Nativo de la isla mediterránea de Córcega en Francia. Es una raza pequeña y generalmente gris. actualmente se estima una población de únicamente 1000 ejemplares.
Miranda	Nativa del noreste de Portugal, tiene una altura máxima de 1.35 m, presenta orejas largas con abundante pelo, cascos grandes, extremidades grandes y fuertes, musculosos, cuello y espalda fuertes. Es la única raza reconocida en Portugal, se distingue de otras razas por su pelo que es más largo.
Burro Negro de Berry	De origen francés. Su altura va de 1.35 a 1.45 m y las hembras el tamaño mínimo es de 1.3 m, es de color negro, parte inferior del hocico y vientre son de color gris.
Razas norteamericanas	Las sociedades de cría de Canadá y Estados Unidos no registran como razas a los burros, son descritas como burros miniatura, estándar y grandes. Se trata de los animales descendientes de los traídos por Cristóbal Colón y que se extendieron de México a Estados Unidos y Canadá. Constituyen el 0.1% de la población mundial de burros.
Provenza	Raza de origen francés, los machos alcanzan una altura de 1.20 a 1.35 m y las hembras de 1.17 a 1.30 m, su color característico es gris paloma que puede variar de pálido a oscuro con tonos rosados.
Burro de los Pirineos	De origen francés, los machos miden de 1.25 a 1.35 m de alzada, las hembras de 1.20 a 1.35 m, el color del pelo puede ser negro brillante, castaño o parcialmente negro. Las hembras pueden ser utilizadas para la producción de leche.
Zamorano-Leonés	Raza española, es una raza de talla grande los machos miden hasta 1.45 m y pesan 370 kg. Son de pelo largo y abundante de color negro o laurel oscuro; vientre, hocico y borde de los ojos de color pálido.
Mamut	Raza norteamericana, descendiente de burros grandes importados desde 1785, usado para la cría de mulas de trabajo. Las razas que participaron en la creación de esta raza fueron el Maltes, Poitou, Andaluz, Mallorquín y Catalán. Los machos alcanzan una altura de 1.42 m y las hembras 1.32 m. Cualquier burro americano que supere estas alturas es inscrito en el Registro Americano de burro Mamut Americano. El burro Mamut Americano más grande registrado ha medido 1.73 m.
Amiatina o Italiano	Burro de la toscana del centro de Italia, es de tamaño medio o estándar la altura máxima que alcanzan es de 1.40 m, el pelaje es de color gris ratón, rayas de cebra en las extremidades. Es una raza fuerte y rustica, ideal para terrenos difíciles.
Asinara	Nativo de Italia, es una raza salvaje, la mayoría de la población es total o parcialmente albina.

Source: https://variedadess.net/razas-de-burros/ (2021). Consulted online 10/10/2021 lives in the center of the country. Meanwhile, wild donkey populations are concentrated in northwestern México, in the states of Durango, Chihuahua, Coahuila, and Sonora (Álvarez-Romero and Medellín, 2005). Worldwide research about their behavior and proper management in production systems (where they are used for different jobs) is still scarce (Velásquez Mosquera *et al.*, 2019; Lagos *et al.*, 2021).

Nowadays, donkeys have become popular in tourist activities known as animal tourism. In the case of Tijuana, Baja California, the celebration of the burro cebra ("zebra donkey") has been held for more than 100 years. Donkeys are painted with stripes to imitate the fur of a zebra during this celebration, which has been recognized as a state heritage since 2014. In the State of Mexico, the Feria Nacional del Burro is held in the municipality of Otumba, in the month of April. The population recognizes this fair as an important cultural affair. The three activities that involve the participation of donkeys are the costumed donkey parade, a donkey race, and polo games. Both states benefit economically, socially, and culturally from these festivities (Quintero-Venegas and Rosales-Estrada, 2020).

The Mexican donkey is remarkable hardy, managing to carry a load of up to 80 kg on its back and pull up to 350 kg. This hardiness is the result of the lack of genetic selection, which has allowed it to maintain its rusticity and consequently to withstand adverse conditions and to survive making the most of the poor-quality forage. All these characteristics makes it a very efficient animal (Pérez, 2019; González, 2021).

General aspects of mules and their arrival to Mexico

Originally the term "mule" was used to name any descendant of two different types of equids (Pascual-Barea, 2016). The first mules were bred in the north and northeast of modern-day Turkey. Known as the oldest human-made hybrid, they originally resulted from the crossing of wild donkeys and mares that shared the same territory (Dave, 2021). Mules are highly versatile and independent animals, like donkeys and unlike horses. They are 1.52 to 1.77 m tall and weigh from 300 to 680 kg. They are long-lived animals, whose life expectancy is 35 to 40 years (Davis, 2019; Dean, 2021). Their characteristics are highly appreciated: thousands of years ago, they were preferred over horses as beasts of burden and for trade activities.

Mules are very efficient hybrid working animals resulting from the cross between a male donkey and a mare (Mosquera *et al.*, 2019). They have a similar height and teeth to horses (Australia, 2019; Dean, 2021). Ninety-nine percent of mules are sterile, as a consequence of their odd number of chromosomes (63). Although, some mules can be crossed with horses or donkeys and their pregnancies can reach their term, their offspring will be weak and sickly. Meanwhile, hinnies are a hybrid resulting from the crossing of a horse with a female donkey (Lozano *et al.*, 2011; Dean, 2021).

Mules arrived to Mexico in the 16th century (Mijares, 2010). In 1495, Christopher Columbus brought donkeys and mares to the "New World" and began breeding mules which the conquistadores used during their expeditions (Dave, 2021). Mules played a major role in the conquest and mining activities. In the 16th century, they were used to transport silver from Mexico City to the port of Veracruz and various goods on both directions of that route. They were used for their ability to move through inaccessible terrain (Smith, 2016). In the 20th century, the mule became an essential means of transportation for different social strata. Its physical characteristics allowed it to adapt to the conditions of the difficult-to-access terrains of the country. This remarkable animal is of great size and vigor. Its typical short but firm step allows it to travel on any type of road, carrying 115 to 200 kg loads (depending on the size of the animal). They have been used to pull wagons and carts —for example, during the boom of the mining industry in several states, including Zacateas (Mijares, 2010). Consequently, they became the beasts of burden of choice (Australia, 2019).

Mules are important for the development of agricultural sustainability in mountain areas (Velásquez Mosquera *et al.*, 2019). Mules are believed to be usually more aggressive than horses and to hinder work; however, if they are worked with a lot of patience and from an early age, a strong bond can be created between human and animal that increases the confidence and security with which both the animal and the handler work (Davis, 2019). They are very intelligent animals that need to be worked with respect to create trust and thus obtain a friendly response. If their trust is obtained, the strong bond that is created means that mules always seek to protect their handlers.

Mule population in Mexico

Currently the mule population in the Americas is approximately 10 million. Mexico is the country with the highest number of mules with 3,287,994 individuals, followed by Brazil with 751,921.8 and China with 428,700 (FAO, 2021; Norris *et al.*, 2021). Norris *et al.* (2021) reports an ongoing decline in the number of mules: from 1961 to 2007, the world population remained around 10 million, but it has decreased to less than 8 million (7,936,976) from 2007 to 2019. For its part, Mexico reached three million mules in 1969 and, according to the information provided by the FAO, the population remained constant until 2019 (Allan, 2021).

CONCLUSIONS

The outlook for donkeys and mules may seem complicated given the lack of interest in their production. Nevertheless, they are very noble animals with highly varied attitudes and surprising intelligence that today do not receive the attention and recognition they deserve, despite their social and economic importance, especially for low-income populations. There are plenty of opportunities to work with donkeys and mules in the world. A renewed interest in their use for different purposes (*e.g.*, work and recreation) has arisen in Mexico in recent years.

REFERENCES

Ali, A.B.A., El Sayed, M.A., McLean, A.K., Heleski, C.R. 2019. Aggression in working mules and subsequent aggressive treatment by their handlers in Egyptian brick kilns—Cause or effect? *J. Vet. Behav.* 29, 95–101. https://doi.org/10.1016/j.jveb.2018.05.008.

Ali, A.B.A., Matoock, M.Y., Fouad, M.A., Heleski, C.R. 2015. Are mules or donkeys better adapted for Egyptian brick kiln work? (Until we can change the kilns). *J. Vet. Behav.* 10, 158–165. https://doi.org/10.1016/j.jveb.2014.12.003.

Allan,K.F. 2021. A Landscaping analysis of working equid population numers in LMICs, with policy recomendations. https://www.thebrooke.org/sites/default/files/Images/Equid_Population_Landscaping_Analysis.pdf.

Alvarado-Arellano, A., Olmos, G., Burden, F., Herenandéz-Gil, M., du Toit, N. 2011. Descripción de una técnica de necropsias en equinos realizada en campo; utilizando un burro (*Equus asinus*) como modelo para su desarrollo. 6.

Álvarez-Romero, J. y R. A. Medellín. 2005. *Equus asinus*. Vertebrados superiores exóticos en México: diversidad, distribución y efectos potenciales. Instituto de Ecología, Universidad Nacional Autónoma de México. Bases de datos SNIB-CONABIO. Proyecto U020. México. D.F. http://www.conabio.gob.mx/conocimiento/exoticas/fichaexoticas/Equusasinus00.pdf.

Australia, G. 2019. Arrieros de México…de mulas, asnos y burros. Consultado: 02/11/2021. https://vocesmexico.com/voces-tematicas/cultura/arrieros-de-mexico-de-mulas-asnos-y-burros/.

Dave, B. 2021. History of the Mule». American Mule Museum. Consultado en Línea: 28/11/2021.

Davis E. Donkey and Mule Welfare. *Vet Clin North Am Equine Pract*. 2019 Dec;35(3):481-491. doi: 10.1016/j.cveq.2019.08.005. PMID: 31672200.

Dean, E. 2021. Donkey vs Mule: What's the Difference? (With Pictures). Consultado en Línea: 27/11/2021: https://petkeen.com/donkey-vs-mule/.

Food and Agriculture Organization of the United Nations - (FAO). FAOSTAT.2021. Tendencias en la ganadería. Consultado en Línea: 27/11/2021. https://www.fao.org/faostat/es/#data/EK.

Food and Agriculture Organization of the United Nations - (FAO). FAOSTAT. 2021. Consultado 20/11/2021. Disponible en: http://faostat.fao.org.

Geor, R.J. (Ed.) 2013. Equine applied and clinical nutrition: health, welfare and performance. Saunders Elsevier, Oxford.

González, R. 2021. La esperanza busca salvar de la extinción al burro mexicano. Milenio Puebla. Consultado: 02/11/2021. https://www.inforural.com.mx/la-esperanza-busca-salvar-de-la-extincion-al-burro-mexicano/.

Kimura, B., Marshall, F., Beja-Pereira, A., Mulligan, C. 2013. Donkey Domestication. *Afr. Archaeol. Rev*. 30, 83–95. https://doi.org/10.1007/s10437-012-9126-8.

Lagos, J., Rojas, M., Rodrigues, J.B., Tadich, T. 2021. Perceptions and Attitudes towards Mules in a Group of Soldiers. *Animals* 11, 1009. https://doi.org/10.3390/ani11041009.

Mayers, A. 2017. Under the skin. Sidmouth (United Kingdom): The Donkey Sanctuary. pp. 1–17.

McLean, A.K., Navas González, F.J., Canisso, I.F. 2019. Donkey and Mule Behavior. *Vet. Clin. North Am. Equine Pract*. 35, 575–588. https://doi.org/10.1016/j.cveq.2019.08.010.

México desconocido. 2020. Burro mixteco, la nueva raza de burro 1005 mexicana. consultado en línea: 01/11/2021. https://www.mexicodesconocido.com.mx/burro-mixteco-la-nueva-raza-de-burro-100-mexicana.html#:~:text=Hoy%2C%20gracias%20al%20trabajo%20de,ser%20designado%20como%20una%20raza.

México desconocido. 2021. El burro mexicano, un animal en peligro de extinción. Consultado en línea: 01/11/2021. https://www.mexicodesconocido.com.mx/burro-mexicano-un-animal-en-peligro-de-extincion.html.

Mijares, I. 2010. Caminos y mercados de México, 1. ed. ed, Serie Historia general / Instituto de Investigaciones Históricas. Universidad Nacional Autónoma de México : Instituto Nacional de Antropología e Historia, México, D.F.

Mitchell, P. 2018. The Donkey in Human History 337. An Archaelogical perspective. Oxford University Press. Reino Unido. ISBN 978-0-19-874923-3. P. 337

Mosquera, J.C.V., Álvarez, J.D.C., Jacometo, C.B. 2019. Morphofunctional characteristics of working mules in mountain areas of the Colombian Central Andes. *Acta Sci. Anim. Sci*. 42, e46379. https://doi.org/10.4025/actascianimsci.v42i1.46379.

Nathional Geographic. 2022. El burro, una especie en extinción en México. consultado en línea: 09/10/2022 https://www.ngenespanol.com/animales/burro-en-peligro-de-extincion-en-mexico/.

Norris, S.L., Little, H.A., Ryding, J., Raw, Z. 2021. Global donkey and mule populations: Figures and trends. *PLOS ONE* 16, e0247830. https://doi.org/10.1371/journal.pone.0247830.

Panorama Agropecuario. 2021. El Desarrollo del Burro Mixteco. Consultado en línea 02/11/2021. https://www.youtube.com/watch?v=jS3C11KsbQg.

Pascual Barea, Joaquín. 2016. «Notas al pasaje de Isidoro sobre el mulo y otros híbridos y sobre transmisión al feto de imágenes vistas por la madre». En Santamaría Hernández, María Teresa, ed. Traducción y transmisión doctrinal de la Medicina grecolatina desde la Antigüedad hasta el Mundo Moderno:

nuevas aportaciones sobre autores y textos. Colección Escuela de Traductores de Toledo, 17 (Cuenca: Universidad de Castilla-La Mancha): 25-52.

Queiroz, M.R., Gameiro, M.B.P., Zanella, A.J. 2021. The population of donkeys and mules in Brazil according to agricultural censuses from 1960 to 2017. *Braz. J. Vet. Res. Anim. Sci*. 58, e174365. https://doi.org/10.11606/issn.1678-4456.bjvras.2021.174365.

Quintero Venegas, G.J., Rosales Estrada, P.T. 2020. Valoraciones éticas de dos prácticas zooturísticas en México, cuestiones sobre geografia de los animales. *PatryTer* 3, 30–41. https://doi.org/10.26512/patryter.v3i5.27014.

Rosenbom, S., Costa, V., Al-Araimi, N., Kefena, E., Abdel-Moneim, A.S., Abdalla, M.A., Bakhiet, A., Beja-Pereira, A. 2015. Genetic diversity of donkey populations from the putative centers of domestication. *Anim. Genet*. 46, 30–36. https://doi.org/10.1111/age.12256.

Seyiti, S., Kelimu, A. 2021. Donkey Industry in China: Current Aspects, Suggestions and Future Challenges. *J. Equine Vet. Sci*. 102, 103642. https://doi.org/10.1016/j.jevs.2021.103642.

Smith, D.C. 2016. The book of donkeys: a guide to selecting, caring, and training. LP, Guilford, Connecticut.

Tadich, T.A., de Aluja, A., Cagigas, R., Huerta, L.A., Galindo, F. 2016. Los niños reconocen las necesidades de los burros de trabajo, en Tulimán, México: Observaciones preliminares 3, 7.

The Donkey Sanctuary. 2017. About donkeys - breeds and cross-breeds. In: Knowledge and advice. Consultado en línea: 10/11/ 2021. https://www.thedonkeysanctuary.org.uk/ what-we-do/knowledge-and-advice/about-donkeys.

Velásquez Mosquera, J.C., Correa Rojas, I., Corrales Álvarez, J.D. 2019. Saberes tradiciones sobre manejo de mulares en el área central-andina de Colombia. *Rev. Univ. Salle* 1, 263–276. https://doi.org/10.19052/ruls.vol1.iss79.14.

Colegio de Postgraduados

Germination test of maize (*Zea mays* L.) seeds in a NaCl solution

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ABSTRACT

Objective: To evaluate the germination behavior and to define the initial vigor of maize seeds.

Methodology: The genetic material consisted of four batches of 20 hybrid seeds each (M-2014, SR-2014, SR-2012, and SR-2011), which were germinated in a NaCl solution (0, 5, 10, and 15 dS m^{-1}) in a germination chamber. A randomized complete block experimental design was used, in which the seed batch and salinity level were the experimental units, with four repetitions each. Seeds with defined radicle and plumule were counted for evaluation.

Results: As a result of the comparison of the three batches from the same environment (production site) and different years, the statistical analysis indicated that the longest-lived seeds have a lower germination percentage. Similarly, when batches from different production environments and the same year were compared, M-2014 obtained the highest average germination percentage (92.19%). When the maximum level of salinity (15 dS m^{-1}) was evaluated, an average 41.25% loss in germination was observed.

Conclusions: The test efficiently discriminated seed batches, based on speed and uniformity in NaCl-based germination, therefore providing acceptable results for the evaluation of the viability and vigor of a seed batch.

Keywords: Hybrid, saline solution, initial seed vigor, physiological quality.

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INTRODUCTION

In order to establish a plantation, around 80% of crops require seeds. In addition, their establishment must be ensured, in such a way that a vigorous and uniform emergence of the seedlings guarantees a good development and a better performance. At the same time, in order to ensure that a seed batch is adequately established in the field, their germination capacity should be determined. In this regard, several tests help to infer their physiological potential; however, the seeds are prone to deterioration for different reasons, which limit their germination. The low germination rate of a given seed batch is associated with early signs of disorganization of the cell membrane: abnormalities can occur in seedlings, when an early death of tissues in different parts of the seed (particularly in meristematic tissues) takes place (Marcos-Filho *et al.*, 2015).

In terms of their viability and vigor, the quality of the seeds is considered a physiological quality and is established during the first stage of crop development. Viability refers to the percentage of seeds that can germinate and develop into a normal plant under optimal conditions. Nevertheless, the full germination of the seed is insufficient for agricultural purposes; In addition, it must be able to germinate in adverse field conditions; this characteristic is known as vigor (Bradford, 2004). Therefore, many researchers consider that the analysis of the physiological quality of a seed batch must include their viability, high germination capacity, and vigor, if they are to establish and produce new individuals.

A wide variety of standardized tests are used to determine the physiological quality of a seed batch, but the standard germination test is the most used. Another way to determine physiological quality is the application of the accelerated aging test, which is used to determine vigor. However, this test may have limitations —*e.g.*, a high moisture content can accelerate the deterioration process of the sample (Ramos *et al.*, 2017).

In this regard, Jianhua and McDonald (1996) proposed the use of saline solutions in seed testing conditions, in order to reduce water absorption and improve the uniformity of the results through the reduction of the incidence of fungi in the samples. In its turn, the use of saline solutions to evaluate seed batches helps to determine the germination capacity and to produce a seedling under stress conditions. In addition, it is indicative of excellent genetic potential, at least at this stage (Bernstein and Ayers, 1953; Pearson *et al.*, 1966). However, Grieve and Suárez (1997), Katembe *et al.* (1998), and Khan and Ungar (1998) point out that a saline medium could inhibit germination and seedling growth, as a consequence of low osmotic potential and ionic toxicity. Specifically, Allen *et al.* (1995) mention that NaCl affects the permeability of plasma membranes and increases the influx of external ions and the efflux of systolic solutes in plant cells.

A successful germination will depend on the transportation of water to the tissues that surround the embryo and on the relationship of the water potential between the seed and the medium. A potential gradient prevents the absorption of water by the seeds; therefore, when the osmotic potential of the solution is more negative than the potential of the embryo cells, germination occurs (Aparecida *et al.*, 2015; Cavalcante and Pérez, 1995; Carvalho and Nakagawa, 2000).

The objective of this work was to evaluate the germination behavior of maize seeds in a saline medium, using a NaCl solution to define the initial vigor.

MATERIALS AND METHODS

This research was carried out in the seed analysis laboratory of the Colegio de Postgraduados-Servicio Nacional de Inspección y Certificación de Semillas, located in Montecillo, Estado de Mexico. The genetic material consisted of four batches of hybrid maize seeds, which were produced in different environments-years (M-2014, SR-2014, SR-2012, and SR-2011).

The test was carried out in a germination chamber, with a controlled environment of 25 °C and 100% relative humidity. Twenty seeds from each batch were placed in Petri dishes, whose base was lined with a "medium pore" filter paper. A NaCl saline solution was used at four levels of electrical conductivity (EC): 0, 5, 10, and 15 dS m^{-1}. To that effect, Maas and Hoffman (1977) pointed out that 100% of the production of the maize crop is affected by an EC of 12 dS m^{-1}. This solution was applied periodically to constantly maintain a $\leq$2mm sheet and activate the imbibition process.

The observation of the seeds began from day one; the germination was considered to have taken place when the radicle and plumule were defined. The counting started on day 4.

A randomized complete block experimental design with four repetitions was used. The analysis of variance and comparison of means were carried out with the LSD (Fisher's least significant difference) test, with an error probability of $\alpha=0.05$, using the SAS OnDemand for Academics (Statistical Analysis System) free online access statistical software.

RESULTS AND DISCUSSION

The result of the experiment showed significant differences among batches (environment-year of production) and salinity level. The interaction between these two factors is not significant (Table 1).

The comparison of means between the batches (environment-year of production) and salinity level (Table 2) shows that the technique (germination test in saline solution) makes a statistically significant contribution to determine the germination of maize seed batches. A comparison of means test was carried out to evaluate the differences of the NaCl solution between batches and salinity level.

In this regard, when the seed is subjected to different salinity levels, its behavior indicates a tendency towards a decrease in the germination percentage, as the concentration of the saline solution increases. When the batches from two different environments and the same production year are compared (M-2014 and SR-2014), the former has the highest average germination percentage (92.19%), which shows that the production environment influences the final quality of a given seed batch. In this regard, McDonald (1999) points out that seed deterioration is different in each species and genotype, as a result of environmental and biological factors; furthermore, this phenomeon is not uniform in every seed or in every batch. Therefore, the environment influences the

Table 1. Analysis of variance of the germination behavior in saline solution of maize seeds produced in different environment-years.

Variation factor	Degrees of freedom	Medium square	(Pr≥F)
Environment-year of production	3	4,304.17	<0.0001
Salinity level	3	1,122.92	<0.0001
Environment-year of production * Salinity level	9	69.44	0.5778
Error	48	82.03	
R^2	0.81		
Variation coefficient (VC %)	12.99		
Average	69.68		

Table 2. Tukey's test between the batch (environment-year of production) and salinity level factors in the germination of maize seeds.

Environment-year of production	**Salinity level (NaCl, dS m^{-1})**				**Germination average (%)**
	0	**5**	**10**	**15**	
M-2014	96.25	96.25	88.75	87.50	92.19 a
SR-2014	76.25	75.00	73.75	61.25	71.56 b
SR-2012	67.50	68.75	53.75	41.25	57.81 c
SR-2011	65.00	65.00	53.75	45.00	57.18 c
Germination average (%)	76.25	76.25	67.50	58.75	
	a	a	b	c	

α: 0.05

development of seeds, particularly when the production environment is different from the environment in which they are sown: the place where the seeds are produced can cause major modifications in their chemical composition (Carvalho, 1979; Mayer and Poljakoff-Mayber, 1982).

Similarly, when the three batches from the same environment and different year are compared (SR-2014, SR-2012, SR-2011), the statistical analysis indicates that longest-lived seeds have a lower germination percentage. These results demonstrate that the aging of a seed batch is reflected in the loss of quality, as mentioned by Marcos-Filho and McDonald (1988): seed aging is a sequential set of biochemical and physiological events that progressively reduce quality, leading to the loss of viability, affecting potential returns, and increasing economic losses. In his turn, Carvalho (1979) mentions that seeds can lose their viability as a result of the mechanical damage that poor handling (post-harvest and storage) could cause on a seed batch.

When the effect of salinity levels is compared, a marked decrease in the germination percentage is observed (at a higher level of salinity). In this sense, the results match the findings of Zahedifar (2013) who experimented with different levels of salinity and reported that the average germination rate of maize seeds decreased significantly as salinity increased. The results of Ramezani and Fatemi-Nik (2013) also match the findings of this study: they reported that an increased concentration of NaCl reduces the germination rate, as a consequence of the increase in osmotic pressure and the reduction of water absorption by maize seeds. Therefore, a delay in water absorption by the seeds is caused by the toxic effects of ions which reduce the permeability of the plasma membrane of the embryo (Laynez *et al.*, 2008).

Zahadifar (2013) recorded a 95% decrease in germination when a 2% concentration of NaCl is applied. In the present experiment, a 41.25% loss in the average germination percentage was observed during the evaluation of the maximum salinity level of 15 dS m^{-1} (1.31% concentration). Therefore, in comparison with the ISTA (2004) standard germination test, this test was also an acceptable tool to evaluate the viability and vigor of a seed batch, because it can efficiently discriminate seed batches based on their germination speed and uniformity.

CONCLUSIONS

In conclusion, the vigor of maize seed differs based on the environment-year of production and its response to the level of salinity: a salinity level of 15 dS m^{-1} enables the representative qualification of the physiological quality of a seed batch. Therefore, the germination test used to define the initial vigor of a seed batch resulting from the application of a saline solution is a quick, effective, and reliable way to quantify the physiological quality of maize seeds.

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REFERENCES

Allen G.J., Wyn-Jones R.G. and Leigh R.A. 1995. Sodium transport measured in plasma membrane vesicles isolated from wheat genotypes with different K^+/Na^+ discrimination traits. *Plant Cell Environ. 18*(2): 105-115 pp. https://doi.org/10.1111/j.1365-3040.1995.tb00344.x

Aparecida D. M., López J. C., Moreira G., G. A., Dias de Souza N. J., y De Oliveira B. C. 2015. Germinación de semillas de zanahoria en diferentes condiciones de salinidad y temperatura. *IDESIA, 33*(4): 41-46 pp. http://dx.doi.org/10.4067/S0718-34292015000400006

Bradford, K.J. 2004. Seed production and Quality. Department of Vegetable Crops. University of California. Davis, California, U.S.A. 134 pag.

Bernstein, L.; Ayers, A. 1953. Salt tolerance of five varieties of carrots. *Proceedings of American Society for Horticultural Sciences, 61*(1): 360-366 pp.

Carvalho, M. N. 1979. Sementes: ciência, tecnologia e produção. Fundação Cargill. 3a. Edição. 429 pag.

Carvalho, N.M. e Nakagawa, J. 2000. Sementes: ciência, tecnologia e produção. Universida de Estadual Paulista, Jaboticabal. 588 pag.

Cavalcante, A.M.B.; Perez, S.C.J.G.A. 1995. Efeito do estresse hídrico e salino sobre a germinação de sementes de *Leucaena leucocephala* (Lam) de Wit. *Pesquisa Agropecuária Brasileiras, Brasília, 30*(1): 281-289 pp.

Grieve, C.M.; Suarez, D.L. 1997: Purslane (*Portulaca oleracea* L.): a halophytic crop for drainage water reuse systems. *Plant and Soil, 192*(1): 277–283 pp. https://doi.org/10.1023/A:1004276804529

ISTA (International Seed Testing Association). (2004). International Rules for Seed Testing. Zurich, Switzerland. 243 pag.

Jianhua, Z.; McDonald, M.B. 1996. The saturated salt accelerated aging test for small-seeded crops. *Seed Science and Technology, 25*(1): 123-131 pp.

Katembe, W.J.; Ungar, I.A.; Mitchell, J.P. 1998. Effect of salinity on germination and seedling growth of two *Atriplex* species (Chenopodiaceae). *Annols of Botany, Volume 82*(2): 167–175 pp. https://doi.org/10.1006/anbo.1998.0663

Khan, M. A. Ungar, I. A. 1998. Seed germination and dormancy of *Polygonum aviculare* L. as influenced by salinity, temperature and gibberellic acid. *Seed Science and Technology, Volume 26*(1): 0–17 pp.

Laynez G., J.A.; Mendez-Natera, J.R.; Mayz-Fifueroa, J. 2008. Efecto de la salinidad y el tamaño de la semilla sobre la germinación y crecimiento de plántulas de maíz (*Zea mays* L.) bajo condiciones de laboratorio. *Revista Especializada en Ciencias Químico - Biológicas, Volumen 11*(1): 17-25 pp.

Maas, E.V.; Hoffman, G.J. 1977. Crop salt tolerance. *Journal and drainage division, Volume 103*(2): 115-134 pp. https://doi.org/10.1061/JRCEA4.0001137

Marcos-Filho J. 2015. Seed vigor testing: an overview of the past, present and future perspective. *Scientia Agricola, Volume 72*(4): 363:374 pp. https://doi.org/10.1590/0103-9016-2015-0007

Marcos-Filho J., McDonald, M.B. 1998. Sensitivity of RAPD analysis, germination and vigour test to detect the intensity of deterioration of naturally and artificially aged soybean seeds. *Seed Science and Technology, Volume 26*(1): 177-237 pp.

Mayer, A.M. and Poljakoff-Mayber, A. 1982. The germination of seeds. Pergamon Press. Third Edition. Oxford, England. 210 pag.

McDonald, M.B. 1999. Seed deterioration: physiology, repair and assessment. *Seed Science and Technology, Volume 27*(1): 177-237 pp.

Pearson, G.A.; Ayers, A.D.; Eberhard, D.L 1966. Relative salt tolerance of rice during germination and early seedling development. *Soil Science, Volume 102*(3): 151-156 pp.

Ramezani, M.; Fatemi-Nik, F. 2013. The effects of seed priming on germination of *Gladiolus alatus*. *Advanced Crop Science, Volume 3*(7): 479-483 pp.

Ramos L., R.; Rockenbach de Ávila, M.; Frosi G., B.; Lopes de Souza C.H., Brandão F., L. 2017. Accelerated aging parameters in the prediction of physiological and sanitary quality of birdsfoot trefoil (*Lotus corniculatus* L.) seeds. *Seed Science, Volume 39*(1): 75-82 pp. https://doi.org/10.1590/2317-1545v39n1169264

Zahedifar, M. 2013. Corn Germination and Seedling Characteristics as Influenced by Seed Priming with Potassium Nano Chelate and Potassium Sulfate Fertilizers under Salinity Stress Conditions. *The Journal of Agricultural Science, Volume 46*(4): 219-229 pp.

Bromatological analysis of annatto (*Bixa orellana* L.) seeds

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ABSTRACT

Objective: The objective of this study was to carry out a bromatological and physicochemical analysis of local annatto seeds and a commercial paste.

Design/methodology/approach: The vegetal material used were local annatto seeds to which physicochemical analysis was carried out to determine ashes (Kirk *et al*., 1996), moisture (weight difference), dry matter (Nielsen, 2019), proteins (Kjeldah, AOAC, 1980), FDA and FDN (Van Soest and Wine, 1967), fat (Soxhlet, 1990). In addition, bromatological analysis was carried out on the samples and the commercial paste (Vázquez, 2001).

Results: The results obtained from the physicochemical analysis varied in ranges, where the M3 sample (dark heart-shaped annatto without filaments) presented the highest values. Regarding the bixin content, the highest percentage with 4.09% was obtained in the M2 sample (heart-shaped red annatto without filaments) and the commercial paste was the lowest with 0.56%.

Limitations on study/implications: The importance of carrying out the bromatological and physical-chemical analysis of the local annatto seeds was to determine which of the local samples and the commercial paste contain the highest amount of bixin. The antioxidant properties of bixin, together with a long shelf life in addition to its well-established safety characteristics, result in a very promising natural coloring agent in the food industry, given that it is an FDA-approved food additive: E160 b.

Findings/conclusions: The physicochemical analyzes varied in ranges, where the M3 sample (dark heart-shaped annatto without filaments) presented the highest values. The highest bixin content was found in the smooth heartwood red variety and the lowest value in the commercial paste.

Keywords: Accesions, food, proximal analysis, bixin.

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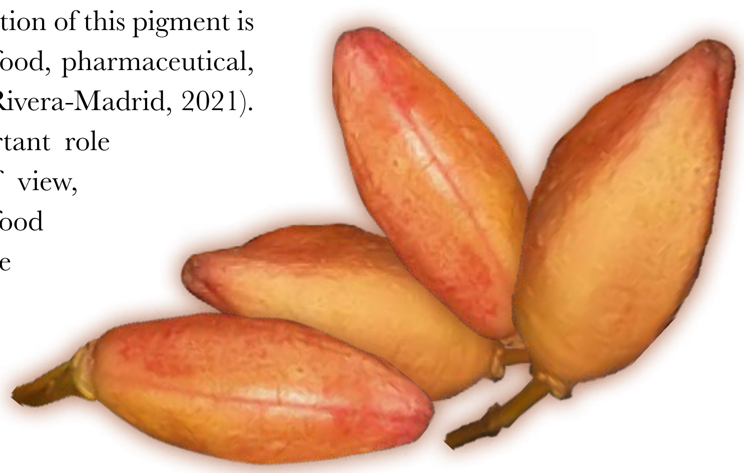
INTRODUCTION

Annatto (*Bixa orellana* L. (Bixaceae) is a perennial shrub whose seeds accumulate a high content of the apocarotenoid pigment bixin, a dye used for its pigmenting qualities, from pre-Columbian times to date. Likewise, bixin is economically and culturally important, since it is consumed in large quantities in Mexico and in the world. The commercialization of this pigment is mainly intended for use in the food, pharmaceutical, textile and cosmetic industries (Rivera-Madrid, 2021). Color, in food, plays an important role from the appearance point of view, which is why colorants as food additives are relevant. They are often used to highlight the natural color of food and others to restore the color



lost during handling for preservation. he latter is the case, for example, with strawberry and pea preserves, which would be unattractive and unappetizing without dyes. Food coloring was already practiced in Roman and Egyptian times (La química y la alimentación, 2018).

Natural dyes are generated by microorganisms, plants, animals, or minerals; economically they represent 940 million dollars in sales in the world market of dyes per year, and due to the consumer's caution for the consumption of products that alter or damage their health, it grows around 4% per year. Natural pigments are those obtained from sources present in nature, used to provide color in some products (Rivera-Madrid, 2021). They are subject to the same quality and toxicological safety testing as synthetics, but the FDA and other government agencies do not require them to be certified for chemical purity, and therefore refer to them as non-certified color additives (Camacaro *et al.*, 2018). In Mexico, there are standards for the food industry that regulate the use of bixin in foods (cheeses, yogurts, meats, creams, margarines, etc.); which establish the permitted doses or concentrations depending on the food and also mention that this colorant does not contain toxic substances that may cause illness to the consumer and that it is of vegetable origin. These Mexican Standards are: NOM-086-SSA1-1994, NOM-120-SSA1-1994, NOM-131-SSA1-1995, NOM-147-SSA1-1996, NOM-185-SSA1-2002, NOM-213-SSA1-2002 (FDA, 2001; Sahaza, 2001). The chemical and biological analysis of food began its operation as a science in the 19th and 20th centuries, with the purpose of making known the characteristics and nutritional value of food (Acero, 2007). The information obtained through bromatology is critical for the assimilation of the factors that condition the properties of foods and, in the same way, for food processing to be safe, nutritious and pleasant for the consumer; since then, the improvement of the quality, quantity and availability of food supply worldwide has been introduced (Acero, 2007). Its importance lies in the economic, hygienic and legislative aspects, which is why it is not enough on its own, since it is essential to complement its execution with other disciplines, taking into account the assessment of the nutritional properties and composition of natural and processed foods and their possible adulterations; chemical analysis of the quantitative content of lipids, carbohydrates, vitamins, proteins and minerals present in the different foods; also, the technical regulation of the sanitary sale of food; as well as industrial production, seriation and transport.

Likewise, investigate the causes that induce and accelerate food alterations and through this develop preventive measures to avoid food from being a vehicle for microorganisms, toxins or any substance harmful to health (Salazar, 2014).

This research contributes to the knowledge of local annatto samples that have potential bixin content in their seeds and can be cultivated. The specific objective of this work was to carry out the bromatological and physicochemical characterization of annatto seeds from two communities in the municipality of Cunduacán, Tabasco and also a commercial paste.

MATERIALS AND METHODS

Annatto seed samples (Figure 1) collected in two communities in the municipality of Cunduacán, Tabasco, Mexico, were used as plant material: six corresponded to the Yoloxochitl 3rd section community and two samples to Monte Grande. The samples were

Figure 1. Annatto samples and seeds collected in two communities in Cunduacán, Tabasco.

placed in paper bags with a capacity of 500 g and labeled; each sample weighed approximately 200 g. These were taken to the Campus Tabasco del Colegio de Postgraduados where they were analyzed in the Animal Science Laboratory and Central Laboratory.

Physicochemical determination and bixin content of annatto seeds

The following parameters were determined for the samples: ashes (Kirk *et al.*, 1996), moisture (weight difference), dry matter (Nielsen, 2019), protein (Kjeldahl, AOAC, 1980), neutral detergent fiber (NDF) and acid detergent fiber (ADF) (Van Soest and Wine, 1967), fats (Soxhlet, 1990) and bixin extraction (Vázquez, 2001). They were performed on each of the original samples and a repeat for each determination. Of the eight samples collected, all were subjected to the seven determinations mentioned above. Except for dry matter, only two varieties were tested. This was due to the fact that when the collection was made, fresh material was needed, and the harvest date had already passed.

Ashes

The methodology developed by Kirk *et al.* (1996) was used. First, the percentage of organic matter (OM) was calculated with the following formula:

$$\%O.M. \equiv \frac{DM - RW}{DM} \times 100$$

(*DM*) Dry Matter; (*RW*) Residual weight.

The percent of ashes was calculated with the following formula:

$$\%C \equiv 100 - \%O.M.$$

%*C*: Percent of Ashes; %*OM*: Percent of organic matter.

Moisture

Moisture determination was only carried out for samples 6 and 8.

$$\%DM = 100 - \%H$$

$P1$: Initial weight; Pf: Final weight.

Dry Matter

The methodology proposed by Nielsen (2019) was used.

$$\%H = \frac{P1 - Pf}{P1} \times 100$$

$\%H$: Percent of moisture; $\%DM$: Percent of Dry Matter.

Protein

The Kjeldahl method was used, and calculations were made using the following formulas:

$$\%P = \frac{(GTHCL)(NHCL)(1.4)}{Pm} \times 6.25$$

$\%P$: Percent of Proteins; $GTHCL$: Total hydrochloric acid consumption; $NHCL$: Normality of hydrochloric acid; PM: Sample weight.

Two adjustment factors were used: Adjustment factor for nitrogen (0.014 mill equivalents multiplied by 100 divided by final sample weight) and Adjustment factor for protein (0.0625 mill equivalents multiplied by 100).

Neutral Detergent Fiber (NDF)

Yields of recovered neutral detergent fiber were expressed as a percentage. Using the following formula:

$$\%NDF = \frac{(bag + sample) - (final\ weight - bag\ weight)}{sample\ weight} \times 100$$

Acid Detergent Fiber (ADF)

The yield calculations were made using the following formula:

$$\%ADF = \frac{(bag + sample) - (final\ weight - bag\ weight)}{sample\ weight} \times 100$$

Fats

The procedure was carried out with the following formula:

$$\%raw\ fat = \frac{M2 - M1}{M} \times 100$$

M: Sample weight; *M*1: flask weight; *M*2: flask weight with fat.

Bixin Extraction

The obtained absorbance was read and the bixin content is calculated using the following formula:

$$\%Bixin = \frac{[A500 + A404 - (0.256 \times A500)]}{286.6 \times 1 \times a} \times 100$$

A=Absorbance of the test solution at the indicated wavelength; 1=Standard cell length (in cm); *a*=Sample concentration (in g/L). 286.6=Absorbance of bixin at 500 nm in chloroform (molar extinction coefficient); 0.256=Factor related to the absorbance of bixin in chloroform at 404 nm and 500 nm.

RESULTS AND DISCUSSION

The results obtained from the physicochemical characterization of the samples are presented in Table 1, where variations in the contents between samples are observed.

Regarding ashes content, sample M1 (7.18%) was higher than all other samples. While the lowest value was found in M6. For moisture content the highest value 41.26% was for M6 from the community of Yoloxochitl and the lowest moisture content in the seeds of sample M8 (33.55%) from Monte Grande. In terms of dry matter, the highest value 66.45% was obtained in M8 and the lowest in sample M6. On the other hand, sample M3

Table 1. Physicochemical characteristics of annatto seeds from two communities in the municipality of Cunduacán, Tabasco, Mexico.

Samples	Ashes	Moisture	Dry Matter	Protein	NDF	ADF	Fats
	%						
S1	7.18	-	-	15.00	74.70	87.97	9.42
S2	4.61	-	-	14.28	65.48	81.03	13.80
S3	5.34	-	-	16.03	75.96	91.05	37.16
S4	5.52	-	-	15.58	65.20	80.46	24.41
S5	5.40	-	-	15.99	75.89	88.70	15.04
S6	3.72	41.26	58.73	15.03	58.66	91.10	23.91
S7	4.32	-	-	14.15	53.31	86.65	28.29
S8	3.84	33.55	66.45	15.13	65.46	90.22	28.29

Note: M1, M2, M3, M4, M6, M7=Samples taken from the community Yoloxochilt 3[a]. Section. M5, M8=Samples taken from the Monte Grande community (Arias-Pérez and De Dios-Durán, 2013).

presented the highest value of 16.03% protein, followed by M5 (15.99%) and the sample with the lowest protein content was M7 with 14.15%. For Neutral Detergent Fiber (NDF) the highest value 75.96% was reached in sample M3 and the lowest value 53.31% in sample M7. For Acid Detergent Fiber (FDA) the highest value 91.10% was obtained in sample M6 and in sample M4 the lowest value with 80.46%. For fat content the highest value (37.16%) was obtained in sample M3 and the lowest value of 9.42% in sample M1.

The results reached in this research for the ash variable are similar to those achieved by authors such as Córdoba (1987), Jaramillo and Muñoz (1992), CNP (2001) and SDIC (2001), who, in studies carried out with annatto of the red variety with abundant filaments, found values that varied from 4.50 to 7.97%. For protein content, the results of the study were superior since the range varied from 14 to 16%, while the previously mentioned authors obtained ranges from 13.00 to 14.24%. The same authors found that moisture content ranged from 8 to 13%. Whereas in the study the two samples M6 and M8 presented moisture contents of 41.26 and 33.35%, respectively. Authors such as, Arias-Pérez and De Dios-Durán (2013) in the same variety of hearty red achiote without filaments obtained moisture percentages of 6.43%; this could be due to the fact that the authors used very small samples of 0.2 g.

Values for NDF ranged from 53.31 to 75.96% and for FDA from 80.46 to 91.10%, which were higher than those obtained by Arias-Pérez and De Dios-Durán (2013), who reported an NDF content of 47.98% and FDA of 39.09%. As the FDA value increases, the digestibility of the seed is reduced as the cell wall is composed of cellulose and lignin (FOSS, 2018). Regarding fat content, the results showed that sample M3 had the highest value of 37.16%, while sample M1 had the lowest content of 9.42%. These results were higher than those achieved by Noguiera-Carvalho *et al.* (2010) who reported only 4.5% of ethereal fat in annatto seeds.

Table 2 shows the results obtained in the extraction of bixin and commercial annatto paste.

Table 2. Bixin extraction performed on annatto seeds and commercial paste.

Sample	Bixin %
Control	0.00
Annatto paste	0.56
M1	3.99
M2	4.09
M3	3.39
M4	3.86
M5	2.00
M6	3.74
M7	1.76
M8	1.08

Note: M1, M2, M3, M4, M6, M7=Samples taken from the community Yoloxochilt 3^{a}. Section. M5, M8=Samples taken from the community Monte Grande. Paste achiote=Sample taken from the annatto paste.

The results of this study differ from those obtained by Valadez-Villarreal *et al.* (2020), who achieved 8.2% bixin in annatto seeds when using an alkaline method for the extraction of the dye. The results varied according to the samples collected. The bixin content was higher (4.09%) in sample M2, the heartwood red variety without filaments, than in the other samples. In samples M5 (green annatto with abundant filaments), M7 (hearty red with few filaments) and M8 (hearty red with abundant filaments) the values found range between 1.08 and 2%, the lowest value corresponds to the annatto paste with 0.56% of bixin. This could be due to the fact that this paste is commercial. INTITEC (2013), recommends for technical requirements in processing plants, that the concentration of bixin should not be less than 2.5%.

CONCLUSIONS

To be approved as an additive, a substance must be well characterized chemically and must pass the toxicological controls established by the corresponding health authorities. In addition, its need must be demonstrated in such a way that its use implies technological advantages and benefits for the consumer. Therefore, the annatto grown in rural communities in Tabasco can be an alternative as raw material of high nutritional value for the food industry and the sample with the highest bixin content should be recommended for its cultivation.

REFERENCES

Acero, G. 2007. Manual de prácticas de Bromatología. 2007. Disponible en: http://www.uaa.mx/centros/cca/MVZ/M/6/Manualdepracticas29-1528.pdf. Fecha de consulta 1 de marzo de 2020.

AOAC. 1980. Oficial Methods of Análisis of the Association of Official Analytical Chemists. Thirteenth edition. 353 p.

Arias-Pérez, I.M. y De Dios-Durán, F.M. 2013. Caracterización morfológica de una muestra local de *Bixa orellana* L., en Tabasco, México. *Revista Agroproductividad, 6*(10):91-97.

Beltrán, R.C.A. 2014. Diseño de un sistema de costos para una empresa agroindustrial de colorantes naturales-achiote. Trabajo de grado de maestría en Contabilidad con mención en Costos y Presupuestos en la Gestión Estratégica. Universidad Nacional Mayor de San Marcos. Facultad de Ciencias Contables. Lima, Perú. 142 pp.

Camacaro, J., Gómez, J., Jiménez, M., Vega C. y Manganiello L. 2018. A liposoluble colorant from Annatto seeds (*Bixa orellana* L.) as an input for food industry. *Revista Ingeniería UC, 2*(25):291-306.

Cordoba, J. A. 1987. El achiote, cultivo, beneficio y posibilidades de exportación. *Revista ESSO Agrícola*. Bogotá, Colombia. pp. 37.

CNP. 2001. Consejo Nacional de Producción. Desarrollo de Productos. Procesos Agroindustriales. Ficha Técnica de Información Nutricional de Achiote. http://www.mercanet.cnp.go.cr/nutriachiote.html.

FDA. 2001. Food and Drugs Administration. Summary of color additives listed for use United States in food, drugs, cosmetics and medical devices. URL: http://www.cfsan.fda.gob/~dms/opa-col2.html. Fecha de consulta, 22 de enero de 2022.

FOSS. 2018. El análisis de la fibra en el pienso animal Fibra cruda, fibra detergente neutra (FDN) y fibra detergente ácida (FDA) - los estándares y las opciones de automatización. Analytics Beyond Measure, e-book. 32 pp.

INTITEC. 2013. 3 Normas Técnicas Peruanas en su versión 2013sobre pinturas, identificación y tecnología nomenclatura de maderas comerciales y de especias y condimentos. Resolución Comisión de Normalización y Fiscalización de Barreras Comerciales no Arancelarias. No. 75-2013 CNB/INDECOPI. Instituto Nacional de Defensa de la Competencia y de la Protección de la Propiedad Intelectual. Lima, Perú.

Jaramillo, M. C. A. y Muñoz M. O. A. 1992. Extracción de colorantes de Achiote. Trabajo de Grado (ingeniero Químico). Medellín. Universidad de Medellín. Facultad de Mina. Departamento de Procesos Químicos. 100 pp.

Kirk. R.S., Egan H. y Sawyer, R. 1996. Composición y Análisis de Alimentos de Pearson. Editorial CECSA. Segunda edición. Grupo Patria Cultural S.A. DE C.V. ISBN 9682612640, 9789682612640. México, D.F. 777 pp.

La Química y la alimentación. 2018. Archivo 28. Foro permanente Química y Sociedad. Federación Empresarial de la Industria Química Española. Madrid, España. 20 pp. Disponible en www.quimicaysociedad.org

Nielsen, S.S. 2019. Food Analysis Laboratory Manual. Third Edition. Springer International Publishing, AG. Gewerbestrasse 11, 6330 Cham, Switzerland. 244 pp.

Nogueira-Carvalho, P.R., Gomes da Silva, M., Gomes Fabri, E., Da Rocha Tavares, P.E., Mello Martines, A. Spatti, L. 2010. Bixin and lipids content in annato seeds from collection of the Instituto Agronómico (IAC). Brangantia, Campinas, 519-524.

NOM-086-SSA1-1994. Norma Oficial Mexicana Bienes y Servicios. Alimentos y bebidas no alcohólicas con modificaciones en su composición. Especificaciones Nutrimentales. SEGOB. Diario Oficial de la Federación. DOF: 22/12/2010.

NOM-120-SSA1-1994. Norma Oficial Mexicana Bienes y Servicios. Prácticas de higiene y sanidad para el proceso de alimentos, bebidas no alcohólicas y alcohólicas. DOF: 28/08/1995.

NOM-131-SSA1-1995. Norma Oficial Mexicana, Bienes y servicios. Alimentos para lactantes y niños de corta edad. Disposiciones y especificaciones sanitarias y nutrimentales. 97 pp.

NOM-147-SSA1-1996. Norma Oficial Mexicana, Bienes y Servicios. Cereales y sus Productos. Harinas de cereales, sémolas o semolinas. Alimentos a base de cereales, de semillas comestibles, harinas, sémolas o semolinas o sus mezclas. Productos de panificación. Disposiciones y especificaciones sanitarias y nutrimentales. Fecha de Publicación: 10 de Diciembre de 1999. 25 pp

NOM-185-SSA1-2002. Norma Oficial Mexicana, Productos y servicios. Mantequilla, cremas, producto lácteo condensado azucarado, productos lácteos fermentados y acidificados, dulces a base de leche. Especificaciones sanitarias. DOF: 16/10/2002

NOM-213-SSA1-2002. Norma Oficial Mexicana, Productos y servicios. Productos cárnicos procesados. Especificaciones sanitarias. Métodos de prueba. DOF: 11/07/2005.

Rivera-Madrid, R. 2021. La cadena de valor del achiote (*Bixa orellana*) detrás del avance científico y tecnológico. Desde el Herbario CICY, 13: 222–226 (18/noviembre/2021). Centro de Investigación Científica de Yucatán, A.C. http://www.cicy.mx/sitios/desde_herbario/ISSN: 2395-8790

Sahaza, D.P. 2001. El Achiote. Disponible en: http://www.unalmed.edu.co/~crsequed/achiote.html. Fecha de consulta, 1 de febrero de 2023.

Salazar, G.A. 2014. Análisis Bromatológico. Disponible en: http://www.corpoica.org.co/sitioweb/Documento/JatrophaContrataciones/Analisisbromatologico.pdf. Fecha de consulta 28 de febrero de 2014.

SDIC (2001). Secretaría de Desarrollo Industrial y Comercial. Achiote. http://oaxaca.gob.mx/sedic/agronegocios/spanish/achiote.html. Fecha de consulta 13 de marzo de 2021.

Soxhlet, F. V. 1990. Official Methods of Analysis AOAC International. 21st Edition, U.S.A. 1-2 p.

A. Valadez-Villarreal, E. López-Hernández, F.L. Ruíz-Santiago, J.A. Hernández-Becerra, R. García-Jiménez. 2020. Aplicación de un extracto de achiote (*Bixa orellana*) en una formulación de un sistema modelo oleoso. Investigación y Desarrollo en Ciencia y Tecnología de Alimentos, 5:609-613.

Soest, P. J. y R. H. Wine. 1967. Use of detergents in the analysis of fibrous feeds. IV. Determination of plant cell-wall constituents. *Assoc. Off. Agr. Chem. Journal*, 50:50-55.

Vázquez, H.C. 2001. Estudio preliminar de la degradación de bixina en polvo en los diferentes tipos de empaques y temperaturas establecidas. Tesis Licenciatura Instituto Tecnológico de Villahermosa. Villahermosa, Tabasco, México.

Neem (*Azadirachta indica* A. Juss) leaves as growth promoter in lambs' diets

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ABSTRACT

Objective: To evaluate the dietary inclusion of neem leaves on the productive and biochemical performance of fattening lambs.

Design/methodology: Forty male lambs were assigned according to a completely randomized design. Treatments consisted of dietary inclusion of neem at 0.0, 2.5, 5.0, and 7.5 g kg^{-1} DM for 35 days.

Results: The inclusion of neem leaf in the diet did not affect the productive performance ($P>0.05$), while protein and energy metabolites were modified ($P\leq0.05$).

Limitations on study/implications: The bioactive compounds present in neem modified the metabolites related to protein and energy metabolism, although these changes did not reflect improvements in the productive performance.

Conclusions: The inclusion of dietary neem (2.5-7.5 g kg^{-1}) has no effect on the productive performance, although it does modify some energy and protein metabolites.

Keywords: bioactive compounds, neem, sheep, protein.

INTRODUCTION

The neem plant (*Azadirachta indica*) is known for its medicinal properties in humans, as antibacterial, antifungal, antiviral, anthelmintic, and hepatoprotector [1]. Meanwhile, in ruminants it is used primarily as fodder and deworming agent [1,2]. Neem contains bioactive compounds such as azadirachtin, nimbidin, nimbidol, salanin and triterpenoid, and depending on the amount ingested, they can be used as growth promoters or become toxic in the diet of ruminants [4].

The assessments that have been made of neem referring to the diet of sheep are based primarily on the use of the seed, the oil and the extract. The incorporation of 3-20% of neem seed paste in lambs' diets did not affect growth, immunity, nutrient digestibility, and meat quality [5,6]. However, the inclusion of 2.5-5% of neem paste in lambs' diets increased the production of microbial protein and improved the efficiency in the use of energy [7], which result in improvements in the consumption of food and weight gain [8]. On the

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other hand, neem seed oil in ewes' diets modified ruminal fermentation, reduced CH_4 production, and increased digestibility of feed and nitrogen metabolism [9]. The addition of the neem leaf extract (50 ppm) in lambs' diets did not have effects on the characteristics of the canal and the lipid composition of meat [10], although it showed high mortality and inhibition of the incubation of *H. contortus* [3].

However, the incorporation of neem leaves in the diet of lambs has not been widely assessed. Neem leaves contain considerable amounts of carotene, proteins (17-20%), and minerals [1,4], in addition to previously mentioned bioactive compounds. Neem leaves in lambs can be used as sole diet [11], complement or replacement of conventional fodders [4,12]. In addition, neem leaves may be used as antibiotic, anthelmintic, and growth promoter when it is added to sheep's diets [2,3]. Supplementation with neem leaf flour (0.5-1%) in sheep's diets increased weight gain [13]. Meanwhile, the consumption of 3 or 6 g of neem leaf in grazing lambs showed improvements in weight gain [14].

The benefits of the inclusion of neem in ruminants' diets thanks to its content in bioactive compounds represent an alternative to improve the productive performance in fattening lambs. Therefore, the objective of this study was to evaluate the dietary inclusion of neem leaves on the productive and biochemical performance of fattening lambs.

MATERIALS AND METHODS

The experimental procedures were carried out following the ethical norms and animal welfare of Colegio de Postgraduados for the use of animals in experimentation. The experiment was conducted in Colegio de Postgraduados, Campus Montecillo, Estado de México (located at 98° 48' 27" W and 19° 48' 23" N), with mean annual temperature of 15.9 °C and 2241 masl.

Forty male lambs (Hampshire×Suffolk) with initial live weight of 33.6+1.9 kg were randomly assigned in a completely randomized design. The treatments consisted in different dietary concentrations of neem leaves (*Azadirachta indica*) of 0.0, 2.5, 5.0 and 7.5 g kg^{-1} DM in its incorporation to a basal diet (metabolizable energy 2.8 Mcal kg^{-1}, raw protein 147.0 g kg^{-1}, non-degradable protein in rumen 65.9 g kg^{-1}, detergent acid fiber 190.8 g kg^{-1}, calcium 7.0 g kg^{-1} and phosphorus 3.4 g kg^{-1}), formulated in agreement with the recommendations by the NRC [15]. The composition of ingredients (g kg^{-1} MS) of the basal diet was the following: sorghum (322.2), corn (210.0), soy paste (116.8), alfalfa hay (156.2), oat straw (103.8), stubble (40.0), sugarcane molasses (40.0), sodium chloride (1.0), and pre-mixture of vitamins and minerals (10.0). The lambs were housed in individual cages equipped with feeding trough and nipple water dispenser. Before the experiment, the lambs were dewormed (Closantil® 5%) and given vitamins (Vigantol® ADE). The feed was offered at 08:00 and 15:00 h attempting to ensure a rejection of 5-15%. Water and food were provided *ad libitum*. The animals had a period of 7 days of adaptation to the experimental diets. The experimental phase lasted 35 days.

The variables assessed were food consumption (CAL), daily weight gain (DWG), food conversion (FC), and final live weight (fLW). The thickness of the dorsal fat and the *Longissimus dorsi* muscle area were measured using an ultrasound (Sonovet 600 Medison, Inc., Cypress, CA, USA) with a transductor of 7.5 Mhz between ribs 12 and 13 on day 35 of the experiment. The yield of the warm canal was evaluated in five animals per treatment after the sacrifice in a commercial facility.

The last day of the experiment, blood samples were collected (5 mL; pre-prandial 08:00) from the jugular vein by puncture, using vacutainer tubes without anticoagulant (BD Vacutainer) and placed immediately in refrigeration (4 °C). The samples were centrifuged (Sigma 2-16 k, Germany) at 3500 g × 20 min to obtain blood serum, which was stored in Eppendorf tubes and kept in a freezer (Sanyo MDF-436, USA) at −20 °C until its analysis. In each sample, the concentrations determined were: total cholesterol (TC, oxidase-peroxidase enzymatic method); triglycerides (enzymatic method); glucose (enzymatic method); total protein (Biuret method); albumin (green bromocresol method); using specific kits from the Spinreact commercial house (Barcelona, Spain) in a visible UV light spectrophotometer (Cary 1- E Varian, USA). Globulin concentration was calculated by difference between total protein and albumin.

Statistical analysis. The experimental design was completely randomized, four treatments and ten repetitions, considering each lamb as an experimental unit. The Shapiro-Wilk and Levene tests were used to verify the normal distribution and homogeneity of the variance of each variable. The data were analyzed with the GLM procedure, and linear orthogonal and quadratic polynomials ($P \leq 0.05$) were used to determine the effect of the neem intake (Statistical Analysis System 2010. Inc. Cary, NC, USA). The initial weight was used as co-variable for weight gain and final weight ($P \leq 0.05$).

RESULTS AND DISCUSSION

Supplementation with neem in lambs did not cause any change in the final weight, daily weight gain, food consumption or food conversion ($P > 0.05$, Table 1). Regarding the thickness of fat, the area of the ribeye chop and the weight of the canal were also not modified ($P > 0.05$, Table 1) from the effect of the addition of neem to the diet. When it comes to blood metabolites (Table 2), this study shows that the concentrations of glucose, urea, total protein, albumins, and the albumin-globulin rate increased linearly ($P \leq 0.05$), while the concentrations of cholesterol and triglycerides decreased in response to the increase of neem in the diet ($P \leq 0.05$).

Although the incorporation of neem leaf flour (3-6 g kg^{-1} MS) in the diet of fattening sheep has shown a positive effect on the daily weight gain [13,14], in this study, no benefit was found in the final weight and the weight gain when evaluating levels of inclusion of 2.5, 5.0 and 7.5 g kg^{-1} DM. There was a similar performance in goats' diets with the incorporation of neem leaves of up to 12%, since there was no decrease in weight gain [16]. In lambs' diets, the inclusion of 2.5-5% of neem paste modified microbial growth, increasing microbial protein and the efficiency in energy use [7], which indicates that potentially the bioactive compounds of neem could improve the use of nutrients, animal health, weight gain, and stimulate feed consumption [8].

In this experiment, a decrease in the consumption of feed was not observed. The incorporation of neem leaves in goats' diets of up to 12% did not show negative effects on the consumption of feed [16]. Also, in goats' diets with a sole diet of neem leaves, a high voluntary consumption of 3.1% of body weight was seen [11]. The principal limitation that has been reported to use neem in sheep's diets lies in its bitter smell and flavor [14]; in the study conducted, this problem could have been reduced because molasses were added to the diet, which could act as flavoring. Now, the period of 7 days of adaptation could help the animal to accept the diets with high neem content, since if there is dietary insufficiency and an adaptation period, it is possible for ruminants to adapt to the flavor of neem [4].

Regarding the assumption that the higher levels of neem used in the evaluation (2.5, 5.0 and 7.5 g kg^{-1} DM) could present an anti-nutritional effect due to the high consumption of its bioactive components (azadirachtin, azadirone, nimbin, nimbidol, solanine and triterpenoid) [6], it is dismissed, since none of the variables evaluated presented a negative effect. With this, it is possible to speculate that the concentration of bioactive compounds of neem present in the diets assessed do not cause any suppressor effect of the productive performance, with which the additional effects of the consumption of such a plant could be assessed [4, 7, 9].

The changes in blood concentrations of total proteins, globulin albumins, and the albumin:globulin rate could be because the neem leaves have noticeable amounts of proteins (17-20%) [1,4], which could alter the concentrations of the biochemical compounds related to the protein metabolism [4]. In a study with fattening lambs where 30% of the *Brassica campestris* straw was replaced with neem leaves, both the digestibility of the protein, the intake of dry matter and protein, and the production of total volatile fatty acids increased [12]. The inclusion of 5% of neem leaves in sheep's diets increased the serum concentration of protein and urea [2]. At the same time, the biochemical compounds present in neem could modify the protein metabolites, since the inclusion of 2.5-5% of neem paste in lambs' diets modified the microbial growth for a higher production of microbial protein [7]. The influence of the bioactive compounds in the changes of protein metabolites could be confirmed with the study where neem seed oil was supplied (20 ml kg^{-1} DM), which does not contain protein altering the nitrogen metabolism in sheep [9]. However, there is evidence that ewes fed with multi-nutritional blocks with 30% of neem leaves did not alter the serum concentrations of protein, globulin, albumins and urea [12].

The increase of glucose concentration in lambs fed with neem (2.5-7 g kh^{-1} DM) could be because ruminants fed with neem leaf [12], oil [9], and paste [7] increase the amount of volatile fatty acids [7,9], primarily the propionic acid that intervenes in gluconeogenesis. When it comes to the reduction of the levels of cholesterol and triglycerides as the neem concentration increased, the use of neem leaves in poultry production is considered as a hypocholesterolemic dietary additive (which inhibits the synthesis blocking the conversion of desmosterol to cholesterol), and hypotriglyceridemic, reducing the content in meat, fat, and blood serum [17].

CONCLUSIONS

The inclusion of neem in the diet (2.5-7.5 g kg^{-1} DM) does not have effects on the productive performance of lambs; however, it does increase some metabolites related to the use of energy and protein, and reduces the serum levels of cholesterol and triglycerides.

Table 1. Dietary addition of neem on the productive performance of lambs.

Item	Neem (g kg^{-1} de MS)				EE	P	
	0.0	2.5	5.0	7.5		Lineal	Cuadrático
Peso vivo inicial, kg	32.90	33.80	33.42	33.10	0.66		-
Peso vivo final, kg	43.77	45.43	44.88	44.57	0.73	0.72	0.11
GDP kg	0.29	0.35	0.33	0.32	0.02	0.61	0.10
Consumo de MS, kg	1.60	1.67	1.63	1.62	0.03	0.81	0.27
CA	5.56	4.94	4.99	5.21	0.30	0.58	0.08
Grasa dorsal mm	3.29	3.26	3.37	3.22	0.11	0.51	0.47
Área de la chuleta, mm^2	1104	1090	1055	1095	35	0.94	0.20
Peso de la canal caliente, %	46.30	45.56	46.34	44.78	0.89	0.35	0.65

DWG: daily weight gain; FC: food conversion; SE: standard error of the mean.

Table 2. Effect of neem on lambs' metabolites.

	Neem (g kg^{-1} de MS)				EE	P	
	0.0	2.5	5.0	7.5		Lineal	Cuadrático
Colesterol (mg dL^{-1})	70.20	69.60	63.80	61.30	1.97	0.01	0.63
Triglicéridos (mg dL^{-1})	41.28	33.56	32.81	34.95	1.87	0.02	0.01
Glucosa (mg dL^{-1})	66.40	74.50	67.40	81.40	4.19	0.05	0.48
Urea (mg dL^{-1})	27.90	30.50	34.40	34.00	1.76	0.01	0.40
Proteína total (g dL^{-1})	7.81	8.74	9.68	10.02	0.18	0.01	0.11
Albuminas (g dL^{-1})	3.62	4.66	5.56	5.82	0.13	0.01	0.39
Globulinas (g dL^{-1})	4.19	4.08	4.12	4.20	0.11	0.89	0.41
Albuminas/globulinas	0.86	1.15	1.36	1.40	0.05	0.01	0.12

SE: standard error of the mean.

REFERENCES

1. Uchegbu, M.; Okoli, I.; Esonu, B.; Iloeje, M. (2011). The grovving importance of neem (*Azadirachta indica* A. Juss) in agriculture, industry, medicine and Environment: A review. *J. Med. Plant Res. 5*(3), 230-245. DOI: 10.3923/rjmp.2011.230.245
2. Adelusi, F.T.; Eniola, O.; Adedokun, S.A.; Alarape, A.B. (2019). Anthelmintic potency of neem (*Azadirachta indica*) leaf meal on west african dwarf (WAD) sheep. *Asian J. Adv. Agric. Res. 11*(2), 1-10. DOI: 10.9734/ajaar/2019/v11i230047
3. Azra, A.; Kaleemullah, M.; Khattak, B.; Asma, N.; Safi, A.U.R.; Qaiser, J.; Afzal, M.; Tahir, U.; Sindhu, Z.U.D.; Farhan, Y. (2019). Comparative efficacy of domestic garlic (*Allium sativum*) and neem (*Azadirachta indica*) against haemonchus contortus in small ruminants. *Appl. Ecol. Environ. Res.* 17, 10389-10397. DOI: 10.15666/aeer/1705_1038910397
4. Adjorlolo, L.K.; Timpong-Jones, E.C.; Boadu, S.; Adogla-Bessa, T. (2016). Potential contribution of neem (*Azadirachta indica*) leaves to dry season feeding of ruminants in West Africa. *Development, 28*(5). http://www.lrrd.cipav.org.co/lrrd28/5/adjo28075.htm
5. Aruwayo, A.; Maigandi, S.A.; Malami, B.S.; Daneji, A.I. (2011). Haematological and biochemical parameters of Uda lambs fed graded levels of alkali-treated neem kernel cake. *Nig. J. Basic Appl. Sci. 19*(2). https://www.ajol.info/index.php/njbas/article/view/73897
6. Rao, S.B.N.; Jash, S.; Dineshkumar, D.; Krishnamoorthy, P.; Elangovan, A. V.; Sivaram, M.; Parthipan, S; Selvaraju, S. (2016). Influence of detoxified neem seed cake on diet digestibility, body weight change, hormonal profiles, immune response and testicular gene expression in male sheep. *Anim. Feed Sci. Technol.* 211, 41-49. DOI: 10.1016/j.anifeedsci.2015.10.008

7. Jack, A.A., Oghenesuvwe, O.; Adewumi, M.K.; Omojola, A.B.; Adegbeye, M.J., Faniyi, T.O.; Salem, A.Z.M.; Elghandour, M.M.Y.; Cuevas-Barragán, C.E.; Barbosa-Pliego, A.; Ekanem, D.E. (2022). Conversion of Neem fruit biomass for rumen manipulation, meat fatty acid profile improvement of rams. *Biomass Convers. Biorefin*. 1-10. DOI: 10.1007/s13399-022-02629-4
8. Jack, A.A.; Adewumi, M.K., Adegbeye, M.J.; Ekanem, D.E.; Salem, A. Z.; Faniyi, T.O. (2020). Growth-promoting effect of water-washed neem (*Azadirachta indica* A. Juss) fruit inclusion in West African dwarf rams. *Trop. Anim. Health Prod. 52*(6), 3467-3474. DOI: 10.1007/s11250-020-02380-w
9. El-Zaiat, H.M., Alqaisi, O.; Sallam, S.M.; Al-Marzooqi, W.S. (2021). Effect of increasing doses of neem (*Azadirachta indica*) seed oil on feed intake, nutrients digestibility, ruminal fermentation and nitrogen utilization of Omani sheep. *Anim. Biotechnol. 33*(7), 1553-1561 DOI: 10.1080/10495398.2021.1917422
10. Webb, E.C.; Hassen, A.; Olaniyi, M.O.; Pophiwa, P. (2022). Effect of dietary inclusion of *Azadirachta indica* and *Moringa oleifera* leaf extracts on the carcass quality and fatty acid composition of lambs fed high forage total mixed rations. *Animals, 12*(16), 2039. DOI: 10.3390/ani12162039
11. Bais, B.; Purohit, G.R.; Dhuria, R.K.; Pannu, U. (2002). Nutritive value of sares and neem leaves in Marwari goats. *Indian. J. Anim. Res. 19*(3), 266-268.
12. Raghuvansi, S.K.S.; Prasad, R.; Mishra, A.S.; Chaturvedi, O.H.; Tripathi, M.K.; Misra, A.K., Sarawat. B.L; Jakhmola, R.C. (2007). Effect of inclusion of tree leaves in feed on nutrient utilization and rumen fermentation in sheep. *Bioresour. Technol. 98*(3), 511-517. DOI: 10.1016/j.biortech.2006.02.008
13. Kumari, P.; Bothra, T.; Narula, H.K.; Jain, D.; Dewna, A.S.; Kumar, U. (2020). Effect of giloy (*Tinospora cordifolia*) and neem (*Azadirachta indica*) on growth performance of Marwari lambs under arid zone. *J. Entomol. Zool. Stud. 8*(3), 849-852. DOI: 10.22271/chemi.2020.v8.i3af.9546
14. Okoruwa, M.I.; Edobor, A.E.; Ogbeide, F.O.; Ikhimioya, I. (2018). Diets containing gliricidia (*Gliricidia sepium*) and neem (*Azadirachta indica*) leaves supplement. *Niger. J. Animal Sci. 20*(1), 106-114. https://www.ajol.info/index.php/tjas/article/view/166082
15. NRC, National Research Council. (2007). Nutrient requirements of small ruminants: sheep, goats, cervids and new world camelids. 7th Ed. National Academy Press. Washington, DC, USA. 384p.
16. Paengkoum, P. (2010). Effects of neem (*Azadirachta indica*) and leucaena (*Leucaena leucocephala*) fodders on digestibility, rumen fermentation and nitrogen balance of goats fed corn silage. *J. Anim. Vet. Adv. 9*(5), 883-886. DOI: 10.3923/javaa.2010.883.886
17. Singh, M.K.; Sharma, R.K.; Singh, S.K. (2017). Neem leaf meal supplementation for profitable poultry production: A review. *Indian J. Poult. Sci. 52*(3), 239-245. DOI : 10.5958/0974-8180.2017.00063.0

Colegio de Postgraduados

Lychee (*Lychee chinensis* Sonn.), composition and possible applications

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ABSTRACT

Objective: to mention the importance of the bioactive compounds and fatty acids (FA) present in lychee, in addition to their possible applications Lychee is a fruit originally from Asia, which has sweet pulp and juice, presents an attractive appearance and pleasant flavor, in addition to high nutritional value, so that it is quite accepted by consumers. Lychee is a great source of bioactive compounds such as tannins and vitamin B1. Because of its functionality, it has various uses as in the preparation of teas and medicinal remedies. Because of the functionality it has, it is important to research this fruit in detail and to find possible applications. Therefore, the objective of this study.

Keywords: Lychee, bioactive compounds, fatty acids, functional foods.

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INTRODUCTION

Presently, it is known that the benefits of a balanced diet are not limited solely to its content in nutrients, but also in seeking other compounds that contribute protection in the presence of oxidative stress and carcinogenesis; they are specially contained in foods of plant origin, the so-called bioactive compounds. Bioactive compounds are components of the foods that influence the cellular activity and the physiological mechanisms with beneficial effects for health (Martínez and Carbajal, 2012).

Fatty acids (FA) are among these bioactive compounds. It is known that these constituents provide biological activity, action mechanisms, structure and molecular formula; they are found in various isolated parts of the plant and fruit. Among them, there is the lychee, which is a fruit of subtropical climate originally from southern China and northern Vietnam (Zhou *et al.*, 2008). It is very popular internationally due to its characteristic flavor and attractive appearance, which makes it a fruit with high commercial value (Cabral *et al.*, 2014). The objective of the study is to understand the importance of the bioactive compounds and FA present in lychee, in addition to their possible applications.

Characteristics of the lychee

The lychee is a fruit originally from Asia that has an attractive appearance and a pleasant flavor, in addition to high nutritional value, so it is quite accepted by consumers (Hajare *et al.*, 2010; Jiang *et al.*, 2004). Because of its functionality, it has various uses, although this functionality differs due to many elements such as the way of harvesting, the cultivar, fertility, and soil type (Lee and Kader, 2000). Lychee is a good source of bioactive compounds. It has a high content of flavonoids, bioactive chemicals, tannins, vitamin B1, good antioxidant activity at the cellular level, and anti-cancer activity (Wen *et al.*, 2015). In addition, it presents a high nutritional value, pharmacological effects such as hepaprotector activity, and antioxidant activity (Bhoophat *et al.*, 2011).

Although some of the properties of lychee are already known, the knowledge about its composition is still limited, since it could have other secondary metabolites in low concentrations, since it is attributed to have many health benefits (Yang *et al.*, 2016).

Bioactive copounds and bioactivity detected in lychee

Presently, it is known that the benefits of a balanced diet are not limited to its nutrient content, but also to other factors that provide protection in face of oxidative stress and carcinogenesis, such as the bioactive compounds contained especially in foods of plant origin. Bioactive compounds are components of foods that influence cellular activity and physiological mechanisms with beneficial effects for health (Martínez and Carbajal, 2012). It is estimated that a mixed diet can contain between 60,000 and 100,000 different bioactive components, potentially effective to reduce the risk of chronic disease, and this is why these elements have been of vital importance in recent studies.

Lychee is a fruit that has a great number of bioactive compounds. Because of these bioactive compounds, the fruit is used in the preparation of teas and medicinal remedies (Wei *et al.*, 2011). It has a high content of flavonoids and lignans detected in the pericarp, in addition to good antioxidant and anti-cancer activity (Wen *et al.*, 2015). It has numerous pharmacological effects such as hepatic protection activity, although it presents reduced levels of phenol compounds. The pulp is mainly composed of carbohydrates and could be responsible for the benefits that it gives to health (Yang *et al.*, 2016).

It has also been found that lychee contains flavonoids (Wen *et al.*, 2014; Jiang *et al.*, 2013), phenolic acids (Wen *et al.*, 2014; Zhang *et al.*, 2013; Sun *et al.*, 2010), proanthocyanidins (Castellain *et al.*, 2014; Xu *et al.*, 2010; Sun *et al.*, 2010), anthocyanins (Li *et al.*, 2012), coumarins (Wang *et al.*, 2011), lignans (Wen *et al.*, 2014; Jiang *et al.*, 2013), chromanes (Lin *et al.*, 2015), sesquiterpenes (Warg *et al.*, 2011; Xu *et al.*, 2010), sterols (Malik *et al.*, 2010), triterpenes (Jiang *et al.*, 2013; Malik *et al.*, 2010), and fatty acids (Xu *et al.*, 2011; Stuart and Buist, 2004). It is known that these constituents provide biological activity, action mechanisms, structure and molecular formula.

Oils and fats

Fatty acids are carboxylic acids with hydrophobic aliphatic chain, and they are divided into four groups according to the number of carbons or length of their chain: volatile, with 2-4 carbons; short chain with 6-10 carbons; medium with 12-16 carbons; and long starting at 16 carbons (FAO, 2008). If they do not have any double links in their molecule, they are called saturated (SFA). When they have double links, they are called unsaturated (MUFA), differentiating between mono-, di-, tri- or poly-unsaturated (PUFA), depending on whether they have one, two, three or more, respectively (Murray *et al.*, 2012; Voet and Voet, 2010; FAO, 2008).

In the case of unsaturated fatty acids, they can be classified depending on the position of the first double bond counting from the terminal methyl group. For the same chemical formula, unsaturated fatty acids can have multiple isomers of structural nature, according to the placement of the double bonds in the chain and depending on whether hydrogens united to the carbon atoms of the double bond are found on the same side (cis) or on both sides (*trans*) of the double bond (FAO, 2008).

Some FA that are consumed have shown to have interesting properties for consumers' health. Among the most important FA, we can count linoleic acid (C18:2, omega-6, AL) and α-linolenic acid (C18:3, omega-3, ALN), and some of long chain such as the docosahexaenoic acid (DHA, 22:6n3) and arachidonic acid (AA, 20:4n6) (Makrides *et al.*, 2011).

Among its positive effects, some that stand out are anti-cancer, anti-atherosclerotic, anti-diabetic (type 2 diabetes) activity, anti-inflammatory and immune activity; in addition to increasing the immune response without muscular catalysis, bone mineralization, lipolysis and anti-lipogenic effects (Molkentin and Giesemann, 2010; Ruggiero *et al.*, 2009; Hunter, 2008). It has also been reported that FA improve the evolution of cardiovascular diseases; and it has been suggested that the consumption in adequate amounts, especially EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid), has a fundamental role on the prevention and/or decrease in risk of developing chronic diseases such as type 2 diabetes, high blood pressure, and even colon cancer (Gutiérrez *et al.*, 2011a; Ozyilkan *et al.*, 2005). It has also been found that conjugated linoleic acid (CLA) has shown to have an important role in regulation of the immune system, as well as in reducing the risk of certain types of cancer and atherosclerosis. Therefore, an increase in the consumption of these fatty acids could have the same beneficial effects for health (Gutiérrez *et al.*, 2011b).

To determine the different fatty acids that make up a sample, various analysis tests must be made and among these there is the fatty acids profile (FAP). The FAP is the study that determines the different fatty acids that are found in a sample analyzed and, thus, to understand or find different acids with nutritional contribution or benefit to the human organism, since numerous experimental studies have suggested a linear relationship between their consumption and their effects on health (Ron, 1986). The FAP of fatty acids has three main stages, which are: extracting or obtaining oil, obtaining methyl esters, and analyzing the sample through instrumental techniques.

Oil extraction methods

When it comes to the oil extraction methods, there are several methods, each with different equipment and reagents. Among the most well-known methods, there is extraction by solvents using the Soxhlet method, extraction by microwave, extraction by ultrasound, extraction with sub and super critical fluids, by hydraulic press, among others (Domingues *et al.*, 2016).

In the extraction by Soxhlet method, different solvents are used such as hexane (Moser *et al.*, 2016; Santos *et al.*, 2013), heptane (NMX-F-490-1987), petroleum ether (Santos *et al.*, 2013; AOAC, 2000), and ethanol (Debora, 2011). The solvent chosen is placed with the sample in question in presence of heating for a certain period of time that can vary between 2-8 h, to obtain the oil and eliminate the solvent by evaporation. The extraction by microwave is also done through solvents, but in this case microwave equipment is used and the oil is obtained by filtering. In the extraction method by sub and super critical fluids, it is done through a continuous flow at high pressure, where carbon dioxide and the solvent is used (mixture of CO_2 and CO_2 with propane), which is pumped to the extractor with a membrane pump with a flow of 1.0-1.5 L/min, at a pressure that ranges between 100-250 bar and a temperature of 35 and 28 °C, respectively, where the extract obtained is cooled in a container for 15 min to eliminate the CO_2 (Illes and Otto, 1997).

Fatty acid extraction methods

After the extraction, there comes a phase of obtaining the methyl esters. This phase consists in the degradation of fatty acids to units of low molecular weight that ease the identification of fatty acids. Among the most well-known methods, there are the methods by Hartman and Lago (1973), Ichihara *et al.*, (1996), Samios *et al.*, (2009), to mention a few. The most frequently used method is the one by Hartman and Lago, where a methanol solution with sodium hydroxide is used and then an esterification reagent; later, filtering is conducted, where the organic phase is dried and filtered using anhydrous sodium sulfate, thus obtaining the methyl fatty acids that will by used to carry out the FAP.

Fatty acids profile

There are different pieces of equipment that may be used to conduct the FAP, among them fine layer chromatography (FLC), high performance liquid chromatography (HPLC), gas chromatography (GC), and other more complex ones such as gas chromatography coupled with mass spectrometry (GC/MS), which help to understand the concentration of each FA present in the sample (Aued-Pimentel *et al.*, 2010).

FLC serves to determine saturated and unsaturated fatty acids (Touchstone, 1995; Nikolova-Damyanova, 2010), and it is a classical method for separation, identification and quantification of fatty acids, due to the simplicity in its use; it is cheaper, with reduced expense in solvents compared to other methods and several samples can done in parallel; in addition, there is the possibility of visualizing the FA with the use of adequate coloring agents (Fuchs *et al.*, 2011).

When it comes to the HPLC, lately it has been used for the determination of FA related to biological, dietary and medicinal samples (Brondz, 2002; Lima and Abdalla, 2002; Rezanka and Votruba, 2002). The use of this equipment dates back to 1950 and its nature is based on its stationary state (solid and liquid). This stationary state is divided into liquid-liquid chromatography, absorption chromatography and reversible phase chromatography, which is a combination of the two previous ones (Brondz, 2002). The parts that are very important to consider in this equipment are the polarity of the stationary phase, the mobile phase, and the chemical structure of the FA. In general, the retention time is proportional to the length of the chain and the number of double bonds present in the FA examined (Rao *et al.*, 1995). This method is used because of its simplicity, reproducibility, and credibility.

GC is the most frequently used method today for the determination of FA. This method uses the sample when it suffers esterification, causing a better yield in the detection of FA. Here, many types of columns can be used that vary according to their properties, such as the polar (commonly used in FA analysis) and non-polar. There are also different types of detectors and among the most common ones in FA analysis, there is the nitrogen detector sensitive to phosphorus, the photometric flame detector, the photoionization detector, the flame ionization detector, and the mass detector (Seppanen-Laakso *et al.*, 2002). However, the most powerful equipment in the detection of FA is the GC/SM, since it detects saturated and unsaturated FA with more accuracy.

In the case of GC/MS, it also serves to determine the molecular weight of the FA present in the sample, in addition to locating double bonds or other functional groups. The basic principle of these instruments is to subject the sample to an impact with electrons, causing the rupture of the aliphatic chain in an indefinite number of fragments, which is why the ramification point and structure of the ring cannot be identified, although the molecular weight of the FA is obtained from the molecular ion and therefore the number of atoms of carbon, hydrogen and oxygen. With this, it can be determined whether the FA is saturated or unsaturated, as well as the ramification point or another constituent (Christie, 1998). With all these data, a better reference of the structures present in the sample can be determined (Dolowy and Pyka, 2014; Christie, 1998).

Recent research

Among the plant samples that have been studied most to determine the FAP, seeds stand out. Table 1 shows some of the seeds about which the FAP has been determined.

As can be seen in the table, the most frequently used extraction method has been Soxhlet, using hexane as solvent. However, there are methods that have better yield in obtaining oils such as the extraction by super critical fluids, by microwave, and the accelerated extraction by solvent which, as has been shown in previous studies (Salvador *et al.*, 2016; Krulj *et al.*, 2016; Ling *et al.*, 2016), present better yield than the method mentioned previously. In addition, these methods reduce the extraction time and energy expense (Golmakani and Rezaei; 2008; Yamini *et al.*, 2008; Kimbaris *et al.*, 2006; Danjanovic *et al.*, 2005).

Another factor that affects the yield of oil extraction is the state of maturity of the seed (Bozdogan and Mungan, 2016; Shan-Shan *et al.*, 2015), since according to some studies, different FA will be found as the fruit is more mature, or FA at higher concentrations in the seed (Tan *et al.*, 2016). Other factors that have an influence are the different types of cultivars (Salvador *et al.*, 2016; Zhang *et al.*, 2016; Shan-Shan *et al.*, 2015), the growth region, and the sample harvest (Zhang *et al.*, 2016), as well as the post-harvest management. These factors should be considered and serve as point of comparison depending on the study or innovation that will be made referring to the study sample, seeking to obtain better results.

Table 1. Determination of FAP of diverse seeds.

Sample	Author/s	Type of extraction	Equipment used	Major acids found (%)	Relevant Data
Jaboticaba	Neuza *et al.*, 2011	Soxhlet (Ethanol)	Gas chromatography	Linoleic (37.01) Palmitic (28.02) Oleic (13.67) α-Linoleic (7.07) Stearic (3.57)	Rare stearic acid.α Linoleic acid is more common from cereal samples.
Water mallow	Moser *et al.*, 2016	Soxhlet (Hexane)	Gas chromatography	Linoleic (53.10) Palmitic (20.70) Oleic (13.70) Malvalic (5.20) Stearic (1.80)	Malvalic acid can be harmful to health.
Palm jelly	Vieira *et al.*, 2016	Soxhlet (Hexane)	Gas chromatography	Lauric (39.17) Oleic (20.73) Capric (11.02) Myristic (7.88) Palmitic (5.19)	This oil can be used as fuel.
Tomato	Botinestean *et al.*, 2012	Cold pressed	Gas chromatography mass spectrometry	Linoleic (54.91) Palmitic (13.52) Oleic (18.85) Octadecenoic (3.54) Linolenic (2.94)	The PAG can tell us the applications that these oils can have.
Olive	Bozdogan y Mungan, 2016	Press	Gas Chromatography	Oleic (62.34-71.53) Palmitic (13.87-16.7) Linoleic (5.02-11.93) Palmitoleic (0.75-1.67) Arachidic (0.56-1.06)	Seed maturation stage is involved in PAG variation.
Nut	Salvador *et al.*, 2016	Low pressure and supercritical fluid extraction	Gas chromatography	Oleic (49-69) Linoleic (19-40) Palmitic (5-11) Stearic (1-6) Linolenic (0-3)	Higher extraction yield was found with the low pressure method. The variety of the sample also influences the PAG.
Amaranth	Krulj *et al.*, 2016	*Soxhlet (petroleum ether) *Supercritical fluids *Accelerated solvent extraction	Gas chromatography	Linoleic (37.29-48.67) Oleic (24.53-33.53) Palmitic (18.68-21.29)	Sample size determines extraction efficiency. Accelerated solvent extraction shows the highest extraction yield.
Pistachio	Ling *et al.*, 2016	*Cold pressing *Microwave	Gas chromatography mass spectrometry	Oleic (55.37-56.05) Linoleic (30.46-31.32) Palmitic (10.95-11.11) stearic (1.05-1.11) Palmitolellic (1.01-1.08)	Most efficient microwave extraction method. Pressing method is more susceptible to oxidation of bioactive compounds.
Lychee	*Gaydou *et al.*, 1993 *Gontier *et al.*, 2000	Soxhlet (petroleum ether)	* Gas chromatography with mass spectrometry * Gas chromatography	Cyclopropanoic (45.10) Palmitic (42.90) Palmitoleic (0.13-1.21) Stearic (0.10-10.50)	Cyclopropanoic acid was found which is usually synthesized by bacteria.

It has been found that most of the samples contain FA such as linoleic, oleic, palmitic and stearic (Zhang *et al.*, 2016; Yang *et al.*, 2016; Vieira *et al.*, 2016; Salvador *et al.*, 2016; Moser *et al.*, 2016; Ling *et al.*, 2016; Krulj *et al.*, 2016; Domingues *et al.*, 2016; Bozdogan and Mungan, 2016; Shan-Sahn *et al.*, 2015; Santos *et al.*, 2013; Botinestean *et al.*, 2012; Neuza *et al.*, 2011; Szentmihalyi *et al.*, 2002; Gontier *et al.*, 2000; Gaydou *et al.*, 1993), in addition to these being found in higher concentration and presenting several positive effects on health (Simopoulos, 2008).

In the case of the oleic acid, values have been found that range from 10.55 to 71.53% (Zhang *et al.*, 2016; Yang *et al.*, 2016; Vieira *et al.*, 2016; Salvador *et al.*, 2016; Moser *et al.*, 2016; Ling *et al.*, 2016; Krulj *et al.*, 2016; Domingues *et al.*, 2016; Bozdogan and Mungan, 2016; Shan-Shan *et al.*, 2015; Santos *et al.*, 2013; Botinestean *et al.*, 2012; Neuza *et al.*, 2011), and canola (63.50%) and olive (71.53%) oils show the highest values. This FA has positive effects at the vascular level, decreasing the systolic and diastolic pressure, and also shows positive effects on the digestive system, in gastric, biliary, pancreatic and intestinal functions (Serra and Aranceta, 2006).

According to studies carried out, levels of linoleic acid that range from 5.02 to 54.91% have been found (Zhang *et al.*, 2016; Yang *et al.*, 2016; Salvador *et al.*, 2016; Moser *et al.*, 2016; Ling *et al.*, 2016; Krulj *et al.*, 2016; Bozdogan and Mungan, 2016; Shan-Shan *et al.*, 2015; Botinestean *et al.*, 2012; Neuza *et al.*, 2011), with the highest value found in tomato (54.91%). This FA has beneficial action on the cardiovascular system (Serra and Aranceta, 2006; Das, 2000), reducing the risk of diseases, and also helping to regulate the production of low-density lipoproteins and to accelerate their dissolution in the organism; and at the same time, helping the healthy function of the brain, hair growth, skin regeneration, energy production, and health of reproductive organs (Patterson *et al.*, 2012; Uauy and Dangour, 2006). Therefore, its consumption is highly advisable for its medicinal properties (Simopoulos, 2008).

When it comes to palmitic acid, it has been found at concentrations that range from 3.60 to 42.90% (Zhang *et al.*, 2016; Yang *et al.*, 2016; Vieira *et al.*, 2016; Salvador *et al.*, 2016; Moser *et al.*, 2016; Ling *et al.*, 2016; Krulj *et al.*, 2016; Domingues *et al.*, 2016; Bozdogan and Mungan, 2016; Shan-Shan *et al.*, 2015; Santos *et al.*, 2013; Botinestean *et al.*, 2012; Neuza *et al.*, 2011; Gontier *et al.*, 2000; 2002; Gaydou *et al.*, 1993) found in lychee seeds (Gaydou *et al.*, 1993; Gontier *et al.*, 2000). This FA has good nutraceutical properties and can be used in the elaboration of functional foods, because it presents medicinal properties such as cardio-protection, anti-inflammatory, among others (Orsavova *et al.*, 2015).

Lastly, stearic acid is one of the most common FA in oils although it is found in low concentrations of 0.10-10.50% (Yang *et al.*, 2016; Salvador *et al.*, 2016; Moser *et al.*, 2016; Ling *et al.*, 2016; Shan-Shan *et al.*, 2015; Neuza *et al.*, 2011; Gontier *et al.*, 2000; Gaydou *et al.*, 1993), with the lychee seed showing the highest value. This FA helps in skin care, so it is widely used in the cosmetic industry and in lubricant additives or the rubber industry (Álvarez and Molina, 1995).

These oils can have various applications in different industries, such as the cosmetic industry (elaboration of soaps, perfumes, fragrances), in rubber production (Álvarez and Molina, 1995), and even in biodiesel production (Domingues *et al.*, 2016; Vieira *et al.*, 2016; Yang *et al.*, 2016). However, where there should be the highest interest is in the food industry, since due to their properties and functionality, these FA can be used in food production as additives, thickening agents, breading product, production of fried foods, giving certain added value to the final product desired.

Medicinal properties of lychee

Thanks to the bioactive compounds found until now in lychee, knowing the contribution that it can give the organism, this fruit has been used medicinally. In Asia many parts of the plant (bark and flowers) are used to relieve throat pain. It is also known that the seed has been used to deal with neurological disorders, hernias, ulcers and intestinal pains (Ahmad and Sharma, 2001). It has been reported that consuming or ingesting moderate amounts of the raw or cooked fruits can relieve cough, in addition to having positive effects in illnesses such as tumors, gland enlargement, cough, diarrhea, stomach ulcers, dyspepsia, obesity, and as treatment to eliminate intestinal parasites (worms). Likewise, the skin tea has been used to cure eruptions caused by smallpox and diarrhea (Castellain *et al.*, 2014; Cohen and Dubois, 2010; Li, 2009).

The tree leaves have also been used for the treatment of flatulence and to detoxify the organism (Wen, 2014). In addition, in the region of Asia and the Pacific, it has been used as promoter to heal wounds (Wiart, 2006).

As had been mentioned before, lychee presents many medicinal properties due to its various nutritional or bioactive compounds (vitamins, dietary fiber, amino acids, oligo elements, linoleic acid, and other unsaturated fatty acids); it can be considered as a functional food and can also be used for the elaboration of functional foods (FFs) (U. S. Department of Agriculture, Agricultural Research Service, 2012; Wall, 2006).

Efects of fa in the human body

Presently, maintaining human health is being sought through the consumption of foods with nutritional contribution that can supply the physiological needs of the organism, and among these foods there are functional foods (FFs). FFs are defined as modified foods or which contain ingredients that increase the welfare of the individual or decrease the risks of diseases, beyond the traditional functionality of ingredients they contain (American Dietetic Association, 2009). Because of the inclusion of FFs, now food also seeks to fulfill the requirements of the diet and the conservation of health (Robertfroid, 2002).

It has been sought for these FFs to be consumed in a healthy and balanced diet, in the same amounts in which typical foods are consumed. The positive effects on health from these foods are because of the bioactive compounds they contain. Among the bioactive compounds, there is AGP n-3 in fish, CLA in ruminant meat, dairy peptides, lutein in egg yolk, and phytochemical substances from vegetables (Biesalski *et al.*, 2009).

Among the positive effects of FFs from the influence of bioactive compounds, there is modulation of the immune system, increasing the phagocytic activity of monocytes and granulocytes, and increasing the levels of cells that secrete antibodies (González-Gross *et al.*, 2004). Positive effects to the digestive system (intestinal tract) have also been found, where these foods help to stimulate the growth of beneficial bifidobacteria. Among these foods there are lactic beverages enriched with fiber, which help to maintain and develop the bacterial flora that is important in the defense mechanisms of the individual. Their intake can prevent or even avoid bacterial translocation (passage of germs of gastrointestinal origin to other tissues such as mesenteric ganglia, liver, spleen and lung), since the final products of fiber are trophic for epithelial intestinal cells, maintaining the balance of the intestinal flora through fermentation and bacterial production of short-chain fatty acids (Sies *et al.*, 2005).

Another positive effect of FFs is as anti-inflammatory agents (they can be used as treatment in acute and chronic inflammation), and this is given by poly-unsaturated fatty acids. This FA is recommended to treat diseases such as rheumatoid arthritis and in the prevention of cardiovascular diseases, due to its beneficial effects against hypercholesterolemia (Platt, 2000). As has been mentioned, due to the properties of FFs, their development promises to increase life quality, especially in risk groups such as children and the elderly. In addition to this, the scientific evidence is the only guarantee that will allow the knowledge of the true functions of FFs, as well as their correct use (Instituto de Nutrición y Transtornos Alimentarios, 2007).

CONCLUSIONS

Lychee presents many bioactive compounds of great biological interest that could be used for diverse applications.

These bioactive compounds can serve to treat various illnesses and diseases, improving the life quality and health of those who consume them.

Many of these compounds should be analyzed further to better determine their functionality.

REFERENCES

Ahmad, S. A. and Sharma, S. C. S. (2001). Fruit and Vegetable Juice Therapy. Pustak Mahal, New Delhi, India.

Álvarez, C. E. y Molina, D. F. (1995). Producción de ácido esteárico a partir de estearina o grasas refinadas mediante el proceso de hidrólisis. Universidad Nacional de Colombia. Santa Fé de Bogotá. Facultad de Ingeniería Química.

American Dietetic Association. (2009). Position of the American Dietetic Association: Functional foods. *Journal American Dietetic Association*. 109: 735-46.

Aued-Pimentel, S., Markievicz M. K. M., Kumagai, E. E., Ruvieri, V. and Zenebon O. (2010). Comparison of gas chromatographic and gravimetric methods for quantization of total fat and fatty acids in foodstuffs. *Quim. Nova. 33*(1): 76-84.

Bhoopat, T., Bhoopat, L., Srichairatanakool, S., Kanjanapothi, D., Taesotikul, T. and Thananchai, H. (2011). Hepatoprotective effects of lychee (*Litchi chinensis* Sonn.): A combination of antioxidant and anti-apoptotic activities. *Journal of Ethnopharmacology. 136*(1): 55-66.

Biesalski, H. K., Dragsted, L. O., Elmadfa, I., Grossklaus, R., Muller, M., Schrenk, D. and Weber, P. (2009). Bioactive compounds: definition and assessment of activity. *Nutrition. 25*(11-12): 1202-1205.

Bozdogan, K. D. and Mungan, B. (2016). Effects of Variety, Maturation and Growing Region on Chemical Properties, Fatty Acid and Sterol Compositions of Virgin Olive Oils. *Journal Ametican Chemical Society*. 93: 1499-1508.

Brondz, I. (2002). Development of fatty acid analysis by high-performance liquid chromatography, gas chromatography, and related techniques. *Analytica Chimica Acta*. *465*(1-2): 1–37.

Cabral, T. A., Morals, C. L. and Pinheiro, S. H. M. (2014). Chemical composition, vitamins and minerals of a new cultivar of lychee (*Litchi chinensis* cv. Tailandes) grown in Brazil. *Fruits*. 69: 425-435.

Castellain, R. C. L., Gesser, M., Tonini, F., Schulte, R. V., Demessiano, K. Z., Wolff, F. R., Delle-Monache, F., Netz, D. J. A., Cechinel-Filho, V., deFreitas, R. A., deSouza, M. M. and Meyre-Silva, C. (2014). Chemical composition, antioxidant and antinociceptive properties of *Litchi chinensis* leaves. *Journal Pharmacy Pharmacology*.66: 1796–1807.

Christie, W.W. (1998). Gas chromatography-mass spectrometry methods for structural analysis of fatty acids. *Lipids*. 33: 343-353.

Cohen, A. and Dubois, L. J. (2010). Raw Food for Everyone: Essential Techniques and 300 Simple to Sophisticated Recipes. Penguin group (USA) Inc., New York, USA.

Danjanovic, B., Lepojevic, Z., Zivkovic, V. and Tolic, a. (2005). Extraction of fennel (*Foeniculum vulgare* Mill.) seeds with supercritical CO_2: Comparison with hydrodistilation. *Food Chemistry*. 92: 143-149.

Das, U. N. (2000). Benefical effect (s). of n- fatty acids in cardiovascular diseases: but, why and how? *Prostag. Leukotr. Ess*. 63: 351-362.

Dolowy, M and Pyka, A. (2014). Chromatographic Methods in the Separation of Long-Chain Mono- and Polyunsaturated Fatty Acids. *Journal of chemistry*. 2015: 1-20.

FAO. (2008). Grasas y ácidos grasos en nutrición humana. Estudio FAO alimentación y nutrición. 91: 204 p.

Fuchs, B., S¨uß, R., Teuber, K., Eibisch, M., and Schiller, J. (2011). Lipid analysis by thin-layer chromatography—a review of the current state. *Journal of Chromatography A. 1218*(19): 2754–2774.

Gaydou, E. M., Ralaimanarivo, A. and Bianchini, J. P. (1993). Cyclopropanoic fatty acids of litchi (*Litchi chinensis*) seed oil. A reinvestigation. *Journal Agritulture Food Chemistry*. 41: 886-890.

Golmakani, M. T. and Rezaei, K. (2008). Comparison of microwave-assisted hydrodistilation with the traditional hydrodistilation method in the extraction of essential oil from *Thymus vulgaros* L. *Food Chemistry*. 101: 1558-1564.

Gontier, E., Boussouel, N., Terrasse, C., Jannoyer, M., Ménard, M., Thomasset, B. and Bourgaud, F. (2000). *Litchi chinensis* fatty acid and diversity: occurrence of the unusual cyclopropanoic fatty acids. *Biochemical Society Transactions. 28*(6): 578-580.

González-Gross, M., Warnberg, J., Álvarez, R., Medina, S. y Marcos, A. (2002). Los Alimentos Funcionales y su relación con el sistema inmune. En: Marcos A, editor. Actualización en Nutrición, Inmunidad e Infección. Madrid: Panamericana. 57-65.

Gutiérrez, R., Vega, S., Radilla, C., Radilla, M., Coronel, S. y Coronado, M. (2011b). The importance of the fatty acids of milk in human nutrition. In: Publishers, N. Y. N. S. (ed.) Milk Production.

Gutiérrez, R., Vega, S., Radilla, C., Radilla, M., Díaz, R., Pérez, J. y Ramírez, M. (2011a). The consumption of fish oil and some benefits in human health. In: Publishers, N. S. (ed.) Fish Oil: Production, Consumption and Health Benefits.

Hajare, S.N., Saxena, S., Kumar, S., Wadhawan, S., More, V., Mishra, B.B., Narayan, P. M., Gautam, S. and Sharma A. (2010). Quality profile of litchi (*Litchi chinensis*) cultivars from India and effect of radiation processing. *Radiat. Phys. Chem*. 79: 994–1004.

Hunter, J. E. (2008). Safety and health effects of trans fatty acids. In Chow, C.K., ed. Fatty Acids in Foods and Their Health Implications. London, CRC Press (Taylor Francis Group). (3rd ed). 757-790.

Instituto de Nutrición y Transtornos Alimentarios (INUTCAM). (2007). Alimentos funcionales, aproximación a una nueva alimentación. Comunidad de Madrid. Salud de Madrid. 238 pp.

Jiang, G., Lin, S., Wen, L., Jiang, Y., Zhao, M., Chen, F., Prasad, K.N., Duan, X. and Yang, B. (2013). Identification of a novel phenolic compound in litchi (*Litchi chinensis* Sonn.) pericarp and bioactivity evaluation. *Food Chemistry*. 136:563–568.

Jiang, Y., Duan, X., Joyce, D., Zhang, Z. and Li, J. (2004). Advances in understanding of enzymatic browning in harvested litchi fruit. *Food Chemistry*. 88: 443–446.

Kimbaris, A. C., Siatis, N. G., Daferera, D. J., Tarantilis, P. A., Papas, C. S. and Polissiou, M. G. (2006). Comparison of destillation and ultrasound assisted extraction methods for the isolation of sensitive aroma compounds from garlic (*Allium sativum*). *Ultrasonics Sonochemistry*. 13: 54-60.

Krulj, J., Brlek, T., Pezo, L., Brkljaca, J., Popovic, S., Zekovic, Z. and Bodroza, S. M. (2016). Extraction methods of *Amaranthus* sp. Grain oil isolation. *Journal Science Food Agriculture*. 96: 3552-3558.

Lee, S. K. and Kader, A. A. (2000). Preharvest and post-harvest factors influencing vitamin C content of horticultural crop. *Postharvest Biology Technology*. 20: 207-220.

Li, W., Liang, H., Zhang, M., Zhang, R., Deng, Y., Wei, Z., Zhang, Y. and Tang, X. (2012). Phenolic profiles and antioxidant activity of Litchi (*Litchi chinensis* Sonn.) fruit pericarp from different commercially available cultivars. *Molecules*. 17: 14954–14967.

Lima, E. S. and Abdalla, D. S. P. (2002). High-performance liquid chromatography of fatty acids in biological samples. *Analytica Chimica Acta. 465*(1-2): 81–91.

Lin, Y., Chang, J., Cheng, S., Wang, C., Jhan, Y., Lo, I., Hsu, Y., Liaw, C., Hwang, C. and Chou, C. (2015). New bioactive chromanes from *Litchi chinensis*. *Journal Agriculture. Food Chemistry*. 63: 2472–2478.

Ling, B., Yang, X and Wang, S. (2016). Physicochemical properties, volatile compounds, and oxidative stability of cold pressed kernel oils from raw and roasted pistachio (*Pistacia vera* L. Var Kerman). *European Journal Lipid Technology*. 118: 1368-1379.

Makrides, M., Collins, C. and Gibson, R. (2011). Impact of fatty acid status on growth and neurobehavioural development in humans. *Maternal and Child Nutrition*. 7: 80-88.

Malik, I., Ahmad, V. U., Anjum, S. and Basha, F. Z. (2010). A pentacyclic triterpene from *Litchi chinensis*. *Natural Product Communications*. 5: 529–530.

Martínez, R. C. y Carvajal, A. A. (2012). Componentes bioactivos de los alimentos. Manual práctico de nutrición y salud. *Conceptos generales*. 31-36.

Molkentin, J. and Giesemann, A. (2010). Follow-up of stable isotope analysis of organic versus conventional milk. *Analytical and Bioanalytical Chemistry*. 398: 1493-500.

Moser, B. R., Seliskar, D. M. and Gallagher, J. L. (2016). Fatty acid composition of fourteen seashore mallow (Kosteletzkyapentacarpos) seed oil accessions collected from the Atlantic and Gulfcoasts of the United States. *Industrial Crops and Products*. 80: 20-26.

Murray, R., Botham, K. M., Kennelly, P. J. and Harper's. (2012). Illustrated Biochemistry, McGraw-Hill Education. Toronto, Canada. 29th edition.

Neuza, J., Bertanha, B. J., Moreno, L. y Debora, M. (2011). Actividad antioxidante y perfil de acidos grasos de las semillas de jabuticaba (*Myrciaria cualiforia* BERG). *Acta biologica colombiana. 16*(2): 75-82.

Nikolova-Damyanova, B. (2010). Fatty acids: silver ion TLC. in Encyclopedia of Chromatography. J. Cazes, Ed. 846–848. Taylor and Francis, New York, NY, USA. 3rd edition.

NMX-F-490-1987. Alimentos para humanos. Determinación de la composición de ácidos grasos a partir de c6 por cromatografía de gases en aceites y grasas. Foods for humans. Oils and fats. Determination of fat acids composition from c6 by gases chromatograpy. Normas mexicanas. Dirección general de normas.

Ozyilkan, O., Colak, D., Akcali, Z. and Basturk, B. (2005). Olive: fruit of peace against cáncer. *Asian Pacific Journal of Cancer Prevention. 6*(1): 77–82.

Platt, R. (2000). Current concepts in optimum nutrition for cardiovascular disease. *Prevention Cardiology*. 3: 83-87.

Rao, M. S., Hidajat, K. and Ching, C. B. (1995). Reversed-phase HPLC: the separationmethod for the characterization and purification of long chain polyunsaturated fatty acids-a review. *Journal of Chromatographic Science. 33*(1): 9–21.

Rezanka, T. and Votruba, J. (2002). Chromatography of very long-chain fatty acids from animal and plant kingdoms. *Analytica Chimica Acta. 465*(1): 273–297.

Robertfroid, M. (2002). Global view on functional foods: European perspectives. *British Journal Nutrition.* 88: S133-8.

Ron, M. (1986). Lípidos marinos y arterioesclerosis. *Medicina interna. 2*(4): 221-225.

Ruggiero, C., Lattanzio, F., Lauretani, F., Gasperini, B., Andres-Lacueva, C. and Cherubini, A. (2009). Ω-3 polyunsaturated fatty acids and immune-mediated diseases: Inflammatory bowel disease and rheumatoid arthritis. *Current Pharmaceutical Design. 15*(36): 4135-4148.

Salvador, A. A., Podesta, R., Block, J. M. and Ferreira, S.R.S. (2016). Increasing the value of pecan nut [Carya illinoinensis (Wangenh) C.Koch] cake by means of oil extraction and antioxidant activityevaluation. *Journal of Supercritical Fluids*. 116: 215-222.

San-Shan, L., Ru-Yu, Y., Li-Guang, Ch., Liang-Sheng, W., Xiang-Hong, H., Li-Jing, W., Xu-Chen, Z. and Hui, D. (2015). Systematic qualitative and quantitative assessment of fatty acids in the seeds of 60 tree peony (Paeonia section Moutan DC.) cultivars by GC–MS. *Food Chemistry*. 173: 133-140.

Sayre, J. K. (2001). Ancient Herb and Modern Herbs: A comprehensive reference guide to medicinal herbs, human ailments, and possible herbal remedies. Bottlebrush Press, Auburn, Washington, USA. 156 p.

Seppanen-Laakso, T., Laakso, I. and Hiltunen, R. (2002). Analysis of fatty acids by gas chromatography, and its relevance to research on health and nutrition. *Analytica Chimica Acta. 465*(1-2): 39–62.

Serra, L. y Aranceta, J. (2006). Nutrición y salud pública: métodos, bases científicas y aplicaciones. 2 ed. Bacerlona, Masson. 826 p.

Sies, H., Stahl, W. and Sevanian, A. (2005). Nutritional, dietary and postprandial oxidative stress. *Journal Nutrition*. 135: 969-972.

Simopoulos, A. P. (2008). The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. *Exp Biol Med*. 233:674–688.

Stuart, L. J. and Buist, P. H. (2004). The absolute configuration of methyl dihydrosterculate: an unusual phyto fatty acid isolated from these ed oil of *Litchi chinensis*. *Tetrahedron: Asymmetry*. 15: 401–403.

Sun, J., Jiang, Y., Shi, J., Wei, X., Xue, S.J., Shi, J. and Yi, C. (2010). Antioxidant activities and contents of polyphenoloxidase substrates from pericarp tissues of litchi fruit. *Food Chemistry*.119: 753–757.

Tan, H., Xiang, X., Tang, J. and Wang, X. (2016). Nutritional functions of the funiculus in *Brassica napus* seed maturation revealed by transcriptome and dynamic metabolite profile analyses. *Plant Molecular Biology*. 92: 539-553.

Touchstone, J. C. (1995). Thin-layer chromatographic procedures for lipid separation. *Journal of Chromatography B: Biomedical Applications. 671*(1-2): 169–195.

U. S. Department of Agriculture, Agricultural Research Service. (2012). USDA National Nutrient Database for Standard Reference, Release 25. Nutrient Data Laboratory. Home Page: ɑhttp://www.ars.usda.gov/ba/bhnrc/ndlɑ

Uauy, R. and Dangour, A. D. (2006). Nutrition and brain development and aging: role of essential fatty acids. *Nutrition Review*. 64: 24-33.

Vieira, B. M., Elicker, C., Nunes, C. F. P., Bairros, A. V., Becker, E. M., Müller, O. D., Piva, E., Fontoura, L. A. M. and Pereira, C. M. P. (2016). The synthesis and characterization of *Butia capitata* seed oil as a FAME feedstock. *Fuel*. 184: 533-535.

Voet, D. and Voet, J. G. Biochemistry, John Wiley and Sons. (2010). New York, USA. 4th edition.

Wall, M. (2006). Ascorbic acid and mineral composition of longan (*Dimocarpus longan*), lychee (*Litchi chinensis*) and rambután (*Nephelium lappaceum*) cultivars grown in Hawaii. *Journal Food Composition Analisys*. 9: 655–663.

Wang, L., Lou, G., Ma, Z. and Liu, X. (2011). Chemical constituents with antioxidant activities from litchi (*Litchi chinensis* Sonn.) seeds. *Food Chemistry*.126:1081–1087.

Wei, B., Huang, L., Teng, J. and Jia M. (2011). Chemical compositional characterization of ten litchi cultivars. *New Technology of Agricultural Engineering* (ICAE), Zibo, Chine.

Wen, L. R., You, L. J., Yamg, W. M., Yang, J. L., Chen, F. and Yiang, Y. M. (2015). Identification of phenolics in litchi and evaluation anticancer cell proliferation activity and intracellular antioxidant activity. *Free Radical Biology and Medicine*. 84: 171-184.

Wen, L., Wu, D., Jiang, Y., Prasad, K.N., Lin, S., Jiang, G., He, J., Zhao, M., Luo, W. and Yang, B. (2014). Identification of flavonoids in litchi (*Litchi chinensis* Sonn.) leaf and evaluation of anticancer activities. *Journal Functional. Foods*. 6: 555–563.

Wiart, C. (2006). Medicinal plants of Asia and the Pacific. CRC Press Taylor and Francis Group, USA. 171 p.

Xu, X., Xie, H., Wang, Y. and Wei, X. (2010). A-Type Proanthocyanidins from Lychee seeds and their antioxidant and antiviral activities. *Journal Agriculture. Food Chemistry*. 58: 11667-11672.

Xu, X., Xie, H., Xu, L. and Wei, X. (2011). A novel cyclopropyl containing fatty acid glucoside from the seeds of *Litchi chinensis*. *Fitoterapia*. 82: 485–488.

Yamini, Y., Khajeh, M., Ghasemi, E., Mirza, M. and Javidnia, K. (2008). Comparison of essential oil composition of *Salvia mirzayanii* obtained by supercritial carbon dioxide extraction hydrodistilation methods. *Food Chemistry*. 108: 341-346.

Yang, B., Nagendra, P. K. and Jiang, Y. (2016). Structure identification of a polysaccharide purified from litchi (*Litchi chinensis* Sonn.) pulp. *Carbohydrate Polymers*. 137: 570-575.

Zhang, R., Zeng, Q., Deng, Y., Zhang, M., Wei, Z., Zhang, Y. and Tang, X. (2013). Phenolic profiles and antioxidant activity of litchi pulp of different cultivars cultivated in Southern China. *Food Chemistry*.136:1169–1176.

Zhang, Z., He, C., Zhu, R., Shen, J., Yu, Y., Peng, Q., Gao, J., Li, Y. and Wang, M. (2016). Seeds oils of five black tartary buckwheat cultivars with biochemical characterization and antioxidant properties. *Journal American Oil Chemistry Society*. 93: 1127-1136.

Zhao, M., Yang, B., Wang, J., Liu, Y., Yu, L. and Jiang, Y. (2007). Immuno modulatory and anticancer activities of flavonoids extracted from litchi (*Litchi chinensis* Sonn.) pericarp. *International Immunopharmacology*. 7: 162–166.

Zhou, B., Chen H., Huang, X., Li, N., Hu, Z., Gao, Z. and Lu, Y. (2008). Rudimentary leaf abortion with the development of panicle in litchi: changes in ultraestructure, antioxidant enzymes and phytohormones. *Scientia Horticulturae* 117:288-292

Synthesis and characterization of polyols made from poultry fat

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ABSTRACT
Objective: Identify the optimal conditions of polyols synthesis from poultry fat by epoxidation and hydrolysis *in situ*.
Design/methodology/approach: Using a statistical design of response surface Box-Behnken type was evaluated the effect of the factors temperature (60, 70 and 80 °C), catalyst (1, 2 and 3 % w/w), molar ratio double bonds:acetic acid (1: 1, 1: 1.5 and 1: 2) and time (4, 6 and 8 h), on the acid index of polyols synthesized.
Results: The FTIR spectra indicated that in mild reaction conditions epoxide groups (827 cm^{-1}) are generated while in severe reaction conditions the formation of OH groups is favored. Therefore, the optimal conditions for the generation of polyol were: 80 °C, 3% w/w catalyst (H_2SO_4), molar ratio double bonds:acetic acid 1:2 and reaction time of 8 h, obtaining a maximum percentage of 78% acidity index, and hydroxyl number of 74 mg KOH/g.
Limitations on study/implications: The removal of the acid medium and water in the reaction was a challenge in polyols with higher acidity index.
Findings/conclusions: The method used of fat extraction, allows to obtain raw material that meets the characteristics to perform the reaction of epoxidation and hydrolysis in a single step to obtain polyol. The most severe conditions of temperature, catalyst concentration, molar ratio of double bonds: acetic acid and reaction time, allowed to obtain the greatest amount of polyol from poultry fat.

Keywords: Hydrolysis, animal fat, epoxidation, agro-industrial waste.

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INTRODUCTION

Recently, there has been an increased interest in renewable raw materials to produce biodegradable materials (Saalah *et al.*, 2017). Polyols are precursors to different polymers, especially polyethers, polyesters, and polyurethanes (Ionescu *et al.*, 2016). However, most commercial polyols are derived from petrochemicals. The most used raw materials for obtaining biopolyols are polysaccharides, carbohydrates, glycerol, and triglycerides, the latter of which can come from either plant or animal sources (Kyriacos, 2020). The most common vegetable oils that have been used for polyol synthesis are soybean (Feng *et al.*, 2017), grapeseed (De Haro *et al.*, 2019), sunflower (Omrani *et al.*, 2016), ricinus (Choi *et al.*, 2015), palm (Yeoh *et al.*, 2020), canola (Kong *et al.*, 2007), which have been transformed through reactions such as ozonolysis, transesterification, epoxidation, and oxirane ring opening (Polaczek *et al.*, 2021). Epoxidation reaction is one of the most convenient methods for obtaining polyols from oils due to the presence of carbon-carbon double bonds in triglycerides, where an epoxy group is introduced and through hydrolysis, the hydroxyl group is formed (Purwanto, 2010). The reaction yield depends on temperature, molar ratio of double bonds:ring-opening reagent, and reaction time. Vegetable oils with a higher degree of unsaturation produce polyols with more hydroxyl groups, resulting in polymers with higher crosslinking density and tensile strength. However, the vegetable oils commonly reported are edible, which generates controversy over their use as raw materials for industrial products. There are reports of trygliceride composition of fish and bovine oils with characteristics suitable for use as raw materials for polymer synthesis (Rohman *et al.*, 2012). In Mexico, more than 3.7 million tons of chicken were produced on 2022 (SIAP, 2022) of which 15% corresponds to fatty residues. Lee and Foglia (2000) mention that chicken fat can be considered a source of unsaturated fatty acids (45-50%), making it attractive for chemical transformations. The objective of this study was to analyze the possibility of using chicken fat for the synthesis of polyols by epoxidation and *in situ* hydrolysis, using a Box-Behnken type response surface statistical design. Four controllable factors were evaluated to achieve maximum conversion: temperature, molar ratio of double bonds:acetic acid, time, and catalyst amount.

MATERIALS AND METHODS

Extraction and characterization of raw material

The chicken fat residues were collected from markets in Tuxtla Gutiérrez, Chiapas, Mexico, solids were removed, and the remaining material was stored at 4 °C until use. Subsequently, the fat was extracted by heating at 90 °C for 6 h. The extracted fat was cooled to room temperature and filtered, cooled, and stored at 4 °C. It was then bleached, deodorized, and fractionated according to the method described by Hernández-Cruz *et al.* (2015). For oxidative stability determination (EN 14112), a biodiesel Rancimat® equipment (Metrohm, Herisau, Switzerland) was used, 3 g of sample were heated to 110 °C and 10 L/h air flow. The kinematic viscosity (ASTM D445) was determined with a SVM 3000® equipment (Anton Paar, Graz, Austria) at 40 °C and 100 °C. To determine the fatty acid profile, the fat was derivatized by alkaline hydrolysis (100 μL) to which 1.0 mL of 0.5 M NaOH was added and heated at 80 °C for 20 min with constant agitation. 1.0 mL of BF_3/MeOH 14% was added and kept at 20 °C for 20 min with constant agitation. Afterwards, the methyl esters were extracted with 1.0 mL of hexane. 1 μL of the extract was injected into a gas chromatograph model 5975 inert XL (Agilent Technologies, Santa Clara, USA), equipped with a DBWax column of 60 m × 0.25 mm × 0.25 μm. The initial temperature in the oven was 150 °C and was maintained for 5 min, then raised to 210 °C (30 °C/min), from 210 °C to 213 °C (1 °C/min), and finally to 225 °C (20 °C/min), it was maintained for 20 min. Helium was used as the carrier gas with a flow of 1 mL/min, with an injector temperature of 250 °C and a split injection ratio of 50:1. An Agilent Technologies model 5975 inert XL mass spectrometer was used to identify the chromatographic signals. The mass spectra were obtained by electron impact ionization at 70 eV. The mass spectra were compared with the HP Chemstation-NIST MS Library version A.00.1995 database. The functional groups were identified by FTIR spectroscopy using a Nicolet™ iS™10 spectrometer (Thermo Scientific, Whaltham, USA). Spectra were obtained after 40 scans in a range of 4000 to 500 cm^{-1} (Jayavani *et al.*, 2017). To obtain the 1H and 13C NMR spectra, a DD2 model NMR equipment from Agilent (California, USA) was used at 500 MHz, 25°C and $CDCl_3$ as solvent. For the determination of acidity index (AOCS Te 1a-64, 2017), 95% neutral ethanol was heated to near boiling and 0.5 M KOH was added until the endpoint was reached. Five grams of sample were added into a 250 mL Erlenmeyer flask, 85 mL of hot neutral 95% ethanol and 0.5 mL of 1% ethanolic phenolphthalein were added, and it was titrated with 0.5 M KOH until the endpoint was reached. The volume of KOH spent was substituted in the following formula (equation 1):

$$\text{Acidity index}\left(\text{mg}\frac{KOH}{g}\text{ of sample}\right)=\frac{V\times M\times 56.11}{P} \quad (1)$$

Where *V* is the volume of *KOH* used during titration (mL); *M* is the molarity of *KOH*, and *P* is the mass of the sample (g).

Epoxidation reaction and obtaining polyols

In the epoxidation reaction and obtaining of polyols, acetic acid (HAc) and hydrogen peroxide (H_2O_2) were added in an equimolar ratio with the moles of double bonds present in the chicken fat. To determine the moles of double bonds, the iodine value was used according to the following formula (equation 2):

$$mol(=)=\frac{IY}{2\times Mi\times 100}\times P \quad (2)$$

Where $mol(=)$ are the moles of double bonds; *IY* is the iodine value obtained, Mi is the molecular weight of iodine (g), and *P* is the weight of the sample (g). For the synthesis of the polyol, 91.47 g of chicken fat was placed in a flask and the calculated amount of HAc and catalyst (H_2SO_4) was added. The mixture was kept under constant agitation and a temperature below 30 °C. Afterwards, H_2O_2 was added dropwise for 30 minutes, the temperature was increased, and the reaction was kept for the times described in the experimental design. Once the reaction time was reached, 5% $NaHCO_3$ was added, followed by washing with 10% $NaHCO_3$, then water, and finally 5% $NaHCO_3$ until neutrality was reached. Finally, 20% w/w Na_2SO_4 was added to the organic pase (Salimon *et al.*, 2014).

Experimental design of the epoxidation reaction

To investigate the impact of operational variables on the response, a Box Behnken type design with response surface was conducted. Catalyst (% w/w, X1), acetic acid (mol/mol(=), X2), reaction temperature (°C, X3), and reaction time (h, X4) were chosen as independent variables. The range of values and coded levels of the variables are presented in Table 1. A polynomial equation was used to forecast the response as a function of independent variables and their interactions.

Table 1. Independent variables in the Box-Behnken design for the epoxidation and ring opening reaction of chicken fat. *(%w/w) relative to the sum of acetic acid and H_2O_2.

Factors		**Levels**		
		−1	**0**	**1**
Catalyst (%p/p)*	X_1	1	2	3
Acetic acid (mol/mol (=))	X_2	1	1.5	2
Temperature (°C)	X_3	60	70	80
Time (h)	X_4	4	6	8

$$Y = \beta_0 + \sum \beta_i x_i + \sum \beta_{ii} x_i^2 + \sum \sum \beta_{ij} x_{ij}$$

Where β_0, β_i, β_{ii} and β_{ij} are constant, linear, square and interaction regression coefficient terms, respectively, and x_i and x_j are independent variables. The STATGRAPHICS Centurion XV was used for multiple regression analysis and analysis of variance (ANOVA). The goodness of fit of the model was evaluated by the coefficient of determination R^2.

Analysis of the epoxidation reaction product and polyol synthesis

To identify the functional groups corresponding to the reaction products, infrared analysis, acidity index, and 1H and 13C nuclear magnetic resonance spectra were performed, as described previously.

Quantification of hydroxyl groups number in polyols

One gram of sample was placed in a 50 mL flask, 10 mL of tetrahydrofuran (THF) was added, mixed, and then 10 mL of catalyst solution (1 g of pyridine in 100 mL of THF) followed by 5 mL of acetylating solution (5 mL of anhydrous acetic acid in 50 mL of THF) were added and stirred for 10 min at 25 °C. Then, 10 mL of hydrolysis solution (20 mL of water in 80 mL of THF) was added and stirred for 30 min. The solution was titrated potentiometrically with 1N ethanolic KOH until pH change. The calculation of the number of hydroxyl groups was obtained using equation 3:

$$ONH = \frac{(V2 - V1 * N * 56.11)}{m} + AN$$

Where ONH is the value of the number of hydroxyl groups; $V2$ is the volume of KOH used in titration of the blank (mL); $V1$ is the volume of KOH used in titration of the sample (mL); N is the normality of KOH; M is the weight of the sample (g), and AN is the acidity index of the sample (Zhang *et al.*, 2015).

RESULTS AND DISCUSSION

Obtaining and characterization of raw material

The extraction yield of chicken fat was 60.71%, similar to that reported by Hernandez-Cruz *et al.* (2020). Additionally, the acidity index was 0.89 mg KOH/g, is similar to that reported for jatropha oil, although lower than that of castor oil (Hazmi *et al.*, 2013). Considering that the acidity index is defined as the number of mg of KOH required to neutralize acidic hydrogen atoms in 1 g of sample, the higher value of acidity index in castor oil is due to the presence of a hydroxyl group in its chemical structure, which is absent in chicken fat and jatropha oil (Valero *et al.*, 2008). With respect to chicken fat oxidative stability (1.42 ± 0.01 h), it is within the range reported for vegetable oils such as sunflower oil (1.1 h), cottonseed oil (1.9 h), jatropha, and moringa oils (1.1 h) (Murru *et al.*, 2021), oils commonly used for the generation of a wide variety of polymers. The value of oxidative stability is indicative of the presence of double bonds that are oxidized under the conditions in which the determination is made (air flow and high temperatures). In other words, the greater the oxidative stability, the greater the number of double bonds, which are the functional groups required for epoxidation, hydrolysis, and obtaining polyol. With respect to kinematic viscosity (66.45 mm^2/s at 25 °C, 36.72 mm^2/s at 40 °C, and 8.09 mm^2/s at 100 °C), it is within the range of viscosities of sunflower oil (64.4 mm^2/s), olive oil (74 mm^2/s), and soybean oil (60.5 mm^2/s) (Quinchia *et al.*, 2010), which are renewable raw materials used for the generation of polyols. Saturated fatty acids with carbon chains generate higher viscosities, and the presence of double bonds causes a decrease in viscosity. In relation to the fatty acid profile, monounsaturated fatty acids (47.37%) are observed in greater proportion, with predominance of oleic acid, followed by saturated fatty acids with 33.17% and majority of palmitic acid, and 19.24% of polyunsaturated fatty acids, with linoleic acid being the principal one. The composition of chicken fat allows for the synthesis of epoxides due to the presence of double bonds that will be oxidized and subsequently hydrolyzed. In the FTIR spectroscopy analysis, the stretching band of unsaturated fatty acid $=C-H$ is observed at 3006 cm^{-1}, and at 2922.15 cm^{-1} and 2853.36 cm^{-1} corresponding to the stretching of Csp3-H bonds. In addition, the ester-type carbonyl group band is observed at 1743.20 cm^{-1}, $C=C$ band at 1656.40 cm^{-1}, and the saturated chain $C-H$ bending vibrations at 1460.36 cm^{-1} and 1372.73 cm^{-1}, as well as the glycerol band at 1157.65 cm^{-1}. The presence of the previously described bands corresponds to the functional groups present in triglycerides, confirming the structure (Wong *et al.*, 2017). The 1H NMR spectrum of the chicken fat (Figure 1) shows signals at 5.2 and 5.5 ppm of the vinyl protons $-C=C-H$; at 4.1 and 4.3 ppm of the glycerol protons $H_2-C-COO-$; the protons adjacent to the carbonyl group ($-CH_2-COO-CH-$) at 2.7 ppm; at 1.3, 1.6, 2 and 2.4 ppm of the CH_2 groups in the carbon chain, and at 0.8 ppm of the $-CH_3$ groups.

Epoxidation and hydrolysis reaction

Figure 2 shows the Pareto chart, where it can be observed that all factors have a statistically significant effect on the acidity index and an R^2 of 76.3708.

The response surface plot (Figure 2B) shows a maximum acid value of 78 mg KOH/g, and optimal reaction conditions of temperature at 80 °C, molar ratio of double bonds to acetic acid at 2, 3% catalyst, and a reaction time of 8 hours, generating the equation from the adjusted model in relation to the response variable:

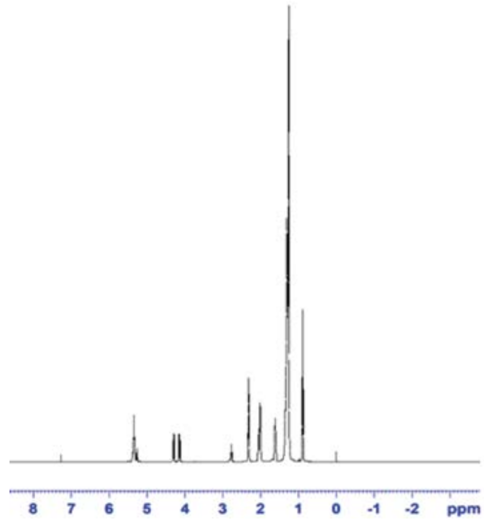

Figure 1. RMN 1H spectrum of chicken fat.

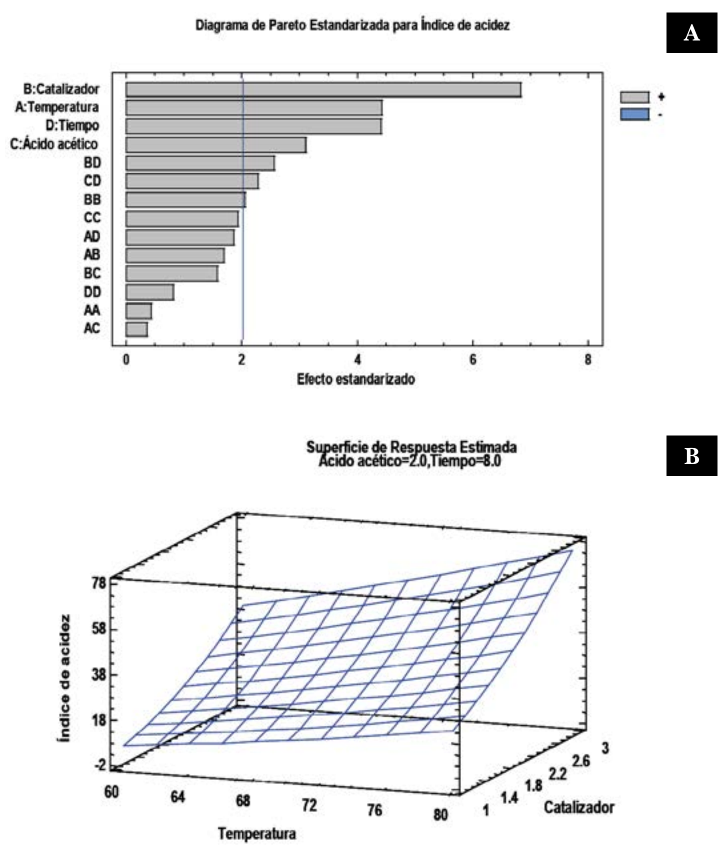

Figure 2. A) Pareto chart obtained from the statistical analysis of the acidity index values, where the effect of temperature, catalyst, acetic acid, and time can be observed. B) Response surface plot for the highest acidity index for the epoxidation and hydrolysis reaction.

$$\text{Acidity index} = 337.045 - 3.40554 * \text{temperature} - 73.0987 * \text{catalyst} - 110.777 * \text{acetic acid} - 35.6272 * \text{time} + 0.0100476 * \text{temperature}^2 + 0.450605 * \text{temperature} * \text{catalyst} + 0.191283 * \text{temperature} * \text{acetic acid} + 0.248587 * \text{temperature} * \text{time} + 4.73505 * \text{catalyst}^2 + 8.40307 * \text{catalyst} * \text{acetic acid} + 3.41624 * \text{catalyst} * \text{time} + 17.8879 * \text{acetic acid}^2 + 6.07902 * \text{acetic acid} * \text{time} + 0.472183 * \text{time}^2$$

As mentioned earlier, the acidity index of chicken fat was 0.89 mg KOH/g, and after the epoxidation and oxirane ring opening reaction, the product increased to 40.63 mg KOH/g, confirming the presence of hydroxyl groups corresponding to the polyol. The FTIR spectrum of the synthesized polyol shows the band at 3432.30 cm^{-1} of OH groups, at 2922.63 cm^{-1} and 2853.90 cm^{-1} of symmetrical stretching C−H, at 1739.90 cm^{-1} of ester carbonyl, at 1460.27 cm^{-1} of asymmetric bending C−H, and at 1097.56 cm^{-1} and 1159.31 cm^{-1} of symmetrical and asymmetrical stretching of C−O−C. The observed bands are similar to the spectrum of the polyol by Ionescu *et al.* (2011), who synthesized polyester polyols from castor oil. In the FTIR spectra at variable temperature (Figure 3A), variable catalyst concentration (Figure 3B), and variable reaction time (Figure 4), it is observed that under low reaction conditions (60 °C, 1% catalyst, 4h), the signal of epoxy groups at 827 cm^{-1}, corresponding to the C−O−C stretching of the oxirane ring, is observed, as well as the absence of signals of hydroxyl groups, confirming the generation of triglyceride epoxides. According to Hazmi *et al.* (2013), the reaction temperature has

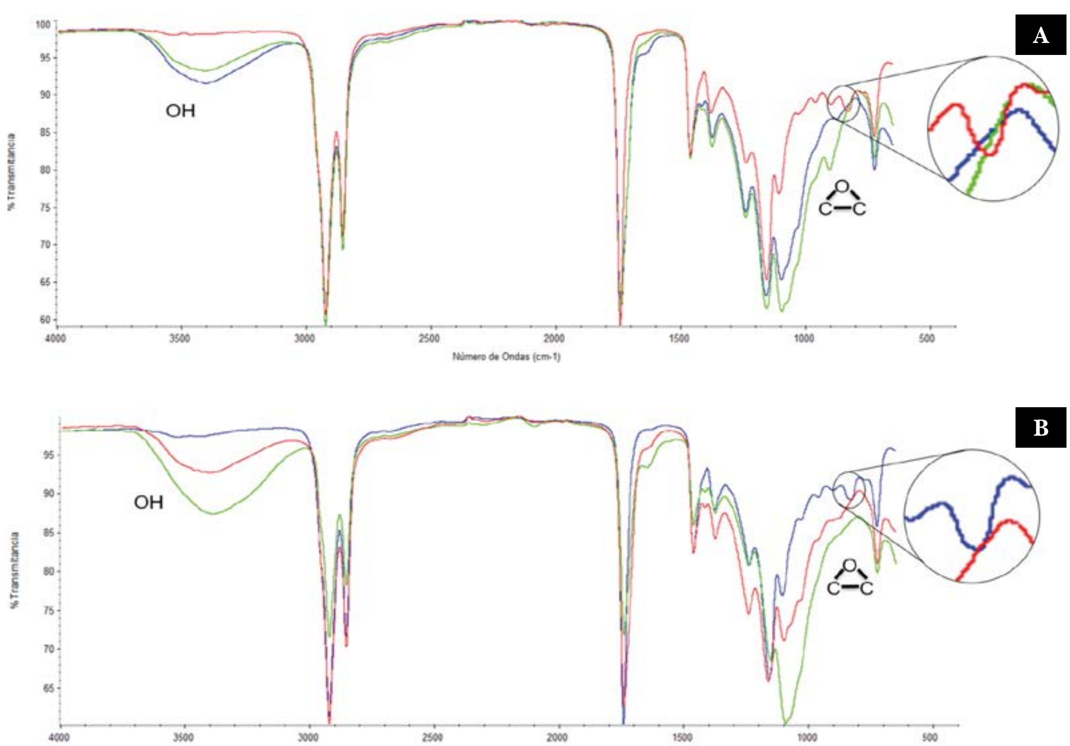

Figure 3. A) FTIR spectrum of the epoxidation reaction and poliol synthesis. Reaction temperature at 60 °C (red), 70 °C (green), and 80 °C (blue). B) FTIR spectrum of the epoxidation reaction and poliol synthesis. Catalyst at 1 mol (blue), 2 mol (red), and 3 mol (green).

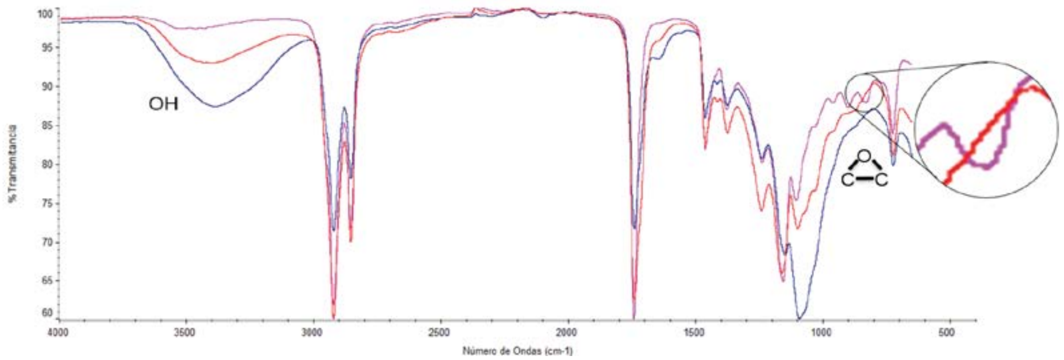

Figure 4. FTIR of the epoxidation reaction and polyol formation. Reaction time: 4 hours (pink), 6 hours (red), and 8 hours (blue).

an impact on the formation of the epoxide group because increasing the temperature can generate ring-opening byproducts.

In the most severe reaction conditions (80 °C, 3% catalyst, and 8h), the signal corresponding to the epoxide group disappears, and there is a higher intensity of signals from hydroxyl groups between 3400-3600 cm^{-1}, indicating the cleavage of the oxirane ring (Milchert *et al.*, 2016).

Thi *et al.* (2015) epoxidized palm oil to synthesize polyols at different reaction times and the same temperature in the presence of water. They observed that at shorter reaction times, there was no oligomerization of the epoxy groups (844 cm^{-1}). However, at longer reaction times, this signal decreased and disappeared at 6 hours, with new stretching bands observed at 3240-3670 cm^{-1} corresponding to the hydroxyl groups. Purwanto *et al.* (2010) reported that the oxirane ring can be hydrolyzed due to the reaction mixture containing H_2SO_4, HAc, and water, which are rich sources of protons that react with the oxygen atoms of the oxirane rings. This can be confirmed by the 1H NMR spectrum of the polyol obtained from the epoxidation and hydrolysis reaction (Figure 5), which shows a signal assigned to the $-C=C-H$ proton group between 5 and 6 ppm.

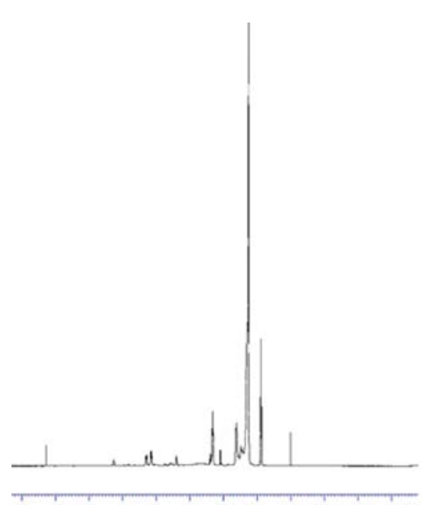

Figure 5. 1H NMR spectrum of the polyol obtained from chicken fat.

Saalah *et al*. (2017) indicate that oligomerization is a side reaction of epoxide ring opening, which occurs when the epoxy group tends to react with acid or water, followed by the dimerization of the already formed hydroxyl compound, where the first step in the reaction is the attack of a proton on the epoxy group, which leads to the formation of hydroxyl and acetyl or formyl groups.

CONCLUSIONS

Chicken fat is a viable source for the formation of polyols through the epoxidation reaction followed by oxirane ring opening. This is feasible due to the fatty acid profile of chicken fat, which confirms the majority presence of palmitic, oleic, and linoleic acids, useful for polyol formation. The optimization of the epoxidation reaction followed by oxirane opening was favorable, demonstrating that all factors influence polyol formation. The higher concentration of catalyst (3 mol), acetic acid (2 mol), temperature (80 °C), and reaction time (8 h) favor the formation of polyols from chicken fat as the formed epoxides are completely hydrolyzed.

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REFERENCES

Saalah, S., Abdullah, L.C., Aung, M.M., Salleh, M Z., Biak, D.R., Basri, M., Jusoh, E.R., Mamat, S. (2017). Physicochemical Properties of jatropha oil-based polyol produced by a two steps method. *Molecules, 22*(4).551. https://doi.org/10.3390/molecules22040551.

Ionescu, M., Polyols. In: Chemistry and technology of polyols for polyurethanes, 2nd ed; iSmithers Rapra Publishing; Smithers Group Company: Shawbury, United Kingdom, 2016; vol. 2, 1-13. https://doi.org/10.1002/pi.2159.

Kyriacos, D. Vegetable Oils, Animal Fats,Carbohydrates and Polyols. In: Biobased Polyols for Industrial Polymers, 1st ed.; Ebnesajjad, S., Scrivener Publishing LLC, Wiley: Beverly, USA, 2020; 1–24. DOI:10.1002/9781119620358.

Feng, Y., Liang, H., Yang, Z., Yuan, T., Luo, Y., Li, P., Yang, Z., Zhang, C. (2017). A solvent-free and scalable method to prepare soybean-oil-based polyols by thiol–ene photo-click reaction and biobased polyurethanes therefrom. *ACS Sustain. Chem. Eng*., 5, 7365–7373. https://doi.org/10.1021/acssuschemeng.7b01672.

De Haro, J., López-Pedrajas, D., Pérez, Á., Rodríguez, J., Carmona, M. (2019). Synthesis of rigid polyurethane foams from phosphorylated biopolyols. *Environ. Sci. Pollut. Res*., 26, 3174–3183. DOI: 10.1007/s11356-017-9765-z.

Omrani, I., Farhadian, A., Babanejad, N., Shendi, H., Ahmadi, A., Nabid, M. (2016). Synthesis of novel high primary hydroxyl functionality polyol from sunflower oil using thiol-yne reaction and their application in polyurethane coating. *Eur. Polym. J*., 82, 220–231. https://doi.org/10.1016/j.eurpolymj.2016.07.021.

Choi, K., Park, S., Oh, K., Kim, S. (2015). Effect of castor oil/polycaprolactone hybrid polyols on the properties of biopolyurethane. *Macromol. Res*., 23, 333–340. https://doi.org/10.1007/s13233-015-3052-y.

Yeoh, F., Lee, C.S.; Kang, Y., Wong, S., Cheng, S., Ng, W. (2020). Production of Biodegradable Palm Oil-Based Polyurethane as Potential Biomaterial for Biomed. *App. Pol*., 12, 1842. DOI:10.3390/polym12081842.

Kong, X., Narine, S. (2007). Physical properties of polyurethane plastic sheets produced from polyols from canola oil. *Biomacromolecules*, 8, 2203–2209. https://doi.org/10.1021/bm070016i.

Polaczek, K., Kurańska, M., Auguścik-Królikowska, M., Prociak A., Ryszkowska J. (2021). Open-cell polyurethane foams of very low density modified with various palm oil-based bio-polyols in accordance with cleaner production, *J. Clean. Prod*., 290, 125875. https://doi.org/10.1016/j.jclepro.2021.125875.

Purwanto, E. 2010. The Synthesis of Polyol from Rice Bran Oil through Epoxidation and Hydroxylation Reactions. Master of Engineering Science, University of Adelaide, Adelaide, Australia, July 2010. Available in https://digital.library.adelaide.edu.au/dspace/bitstream/2440/64753/8/02whole.pdf.

Rohman, A., Triyana, K., Sismindari, Erwanto, Y., Differentiation of lard and other animal fats based on triacylglycerols composition and principal component analysis. *Int. Food Res. J.*, (2012), 19 (2), 475–479, http://www.ifrj.upm.edu.my/19%20(02)%202012/(14)IFRJ-2012%20Rohman.pdf.

Agri-Food and Fisheries Information Service, (2022). Available in http://infosiap.siap.gob.mx/repoAvance_siap_gb/pecResumen.jsp

Lee, K., Foglia, T. Fractionation of Chicken Fat Triacylglycerols: Synthesis of Structured Lipids with Immobilized Lipases. (2000). *J. Food Sci., 65*(5), 826–831. doi:10.1111/j.1365-2621.2000.tb13595.x.

Hernández-Cruz, M., Meza-Gordillo, R., Torrestiana-Sánchez, B., Rosales-Quintero, A., Ventura-Canseco, L., Castañón-Gonzáles, J. (2017). Chicken fat and biodiesel viscosity modification with additives for the formulation of biolubricants. *Fuel*, 198, 42–48. https://doi.org/10.1016/j.fuel.2016.12.039.

Hernández Cruz, M. (2015). Modificación de la viscosidad de grasa de pollo-biodiesel con aditivos, para la formulación de biolubricantes. Maestría en Ciencias en Ingeniería Bioquímica. Instituto Tecnológico de Tuxtla Gutiérrez, Chiapas. Tuxtla Gutiérrez, Chiapas, México.

Jayavani, S., Sunanda, S., Varghese, T., Nayak, S. (2017). Synthesis and characterizations of sustainable polyester polyols from non-edible vegetable oils: Thermal and structural evaluation. *J. Clean. Prod.*, 162, 795–805. https://doi.org/10.1016/j.jclepro.2017.06.040.

Hernández-Cruz, M. (2020). Desarrollo Biotecnológico De Biolubricantes A Partir De Grasa De Pollo. Doctorado en Ciencias. Instituto Tecnológico de Tuxtla Gutiérrez Chiapas.

Salimon, J., Abdullah, B., Yusop, R., Salih, N. (2014). Synthesis, reactivity and application studies for different biolubricants. *Chem. Cent. J., 8*(1), 1–11. https://doi.org/10.1186/1752-153X-8-16.

Zhang, C., Madbouly, S., Kessler, M. (2015). Biobased polyurethanes prepared from different vegetable oils. *ACS App. Mat. Interfaces, 7*(2), 1226–1233. https://doi.org/10.1021/am5071333.

Valero, M., Pulido, J., Ramírez, Á., Cheng, Z. (2008). Síntesis de poliuretanos a partir de polioles obtenidos a partir del aceite de higuerilla modificado por transesterificación con pentaeritritol. *Química Nova, 31*(8), 2076–2082. https://doi.org/10.1590/S0100-40422008000800031.

Murru C., Badía-Laíño R., Díaz-García M. (2021) Oxidative Stability of Vegetal Oil-Based Lubricants, ACS Sustain. *Chem. Eng. 9*(4), 1459-1476. DOI: 10.1021/acssuschemeng.0c06988.

Quinchia, L., Delgado, M., Valencia, C., Franco, J., Gallegos, C. (2010). Viscosity modification of different vegetable oils with EVA copolymer for lubricant applications. *Ind. Crops Prod., 32*(3), 607–612. https://doi.org/10.1016/j.indcrop.2010.07.011.

Wong, J., Aung, M., Lim, H., Md Jamil, S. (2017). Spectroscopic Analysis of Epoxidised Jatropha Oil and Acrylated Epoxidised Jatropha Oil. *Pertanika J. Trop. Agric. Sci., 40*(3), 435–447.

Milchert, E., Malarczyk-Matusiak, K., Musik, M. (2016). Technological aspects of vegetable oils epoxidation in the presence of ion exchange resins: A review. *Polish J. Chem. Technol., 18*(3), 128–133. https://doi.org/10.1515/pjct-2016-0059.

Thi, T., Hoang, K., Kim, I. (2015). Epoxidation and Ring-Opening of Palm Oil to Produce High-Functionality Polyols. *Australian J. Basic App. Sci., 9*(8), 89–93.

Competitiveness of mexican pecans [*Carya illinoinensis* (Wangenh) K. Koch] and almonds [*Prunus dulcis* (Mill.) D. A. Webb] in the international market

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ABSTRACT

Objective: Measurement and analysis of the competitiveness of Mexican pecan nuts (*Carya illinoinensis*) and almonds (*Prunus dulcis*) in the international market with respect to the United States, during the period 2012-2021.

Design/Methodology/Approach: The Revealed Comparative Advantage Index was used to identify the factors that influence its performance and propose strategies to improve its market position.

Results: Mexican pecans to the United States are competitive (0.68 to 0.94) over the years. The IVCR of Mexican pecans to the United States remain consistently above zero (0.68 to 0.94) over the years.

Study Limitations/Implications: Information on the trade of Mexican pecan nuts and almonds may be limited, making it difficult to calculate the IVCR.

Findings/Conclusions: Mexican pecan nut shows favorable competitiveness in the U.S. market while almond its competitiveness is limited, it is not competitive with respect to the market.

Keywords: Production, Competitiveness, Revealed Comparative Advantage Index.

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INTRODUCTION

Nowadays, food production faces a very important challenge: to produce enough high-quality food to supply the growing world population. According to Urrea and Urzúa (2016), pecans are just one of more than 20 existing nut species and one of the 13 nut species native to the USA. Pecans (*Carya ilinoinensis*) are obtained from the pecan tree. They are considered as the most important of all native American nut species and, as a result of their large amount of healthy nutrients, they are consumed by both wild animals and humans (Urrea and Urzúa, 2016).

Both pecans and almonds are two different types of nutraceuticals dry, as a consequence of their nutritional properties and health benefits (Brambila, 2006). In addition, they are extensively used by the food industry, especially in baking and ice cream preparation.

In recent decades, the pecan industry in Mexico has experienced a remarkable growth. According to data from the Servicio de Información Agroalimentaria y Pesquera (SIAP), pecan production in Mexico reached 139,000 tons in the 2019-2020 agricultural cycle, which accounts for a 10% increase, compared with the previous cycle (SIAP, 2020). Likewise, data from SIACON (2023) indicate that 143,721.55 hectares of pecans were planted in 2021, reaching a production of 133,195.39 tons. Out of this total, 8,275.84 tons were exported.

In addition, FAOSTAT (2023) recorded that 33 hectares were used to grow and harvest shell almonds in 2021, resulting in a production of 47.71 tons.

According to Porter (1980), "the competitiveness of a country is defined by the productivity with which it uses its human, economic, and natural resources" (Mathews, 2009). Suñol (2006) points out that the commonly accepted theory of competitiveness refers to the idea that, in poorly developed economies, there is a need to create productive factors and skills.

Meanwhile, Ramírez (2006) mentions that "competitiveness is the ability of economic agents to take advantage of favorable scenarios that usually arise in the world of economics" and that "from the beginning, competitiveness has been linked to international trade and understood as the ability of a given nation to successfully insert itself into the international market." In addition, the Global Competitiveness Report 2010-2011 defines competitiveness "as the set of institutions, policies, and factors that determine the level of productivity of a country" (Bonilla, 2012).

Adam Smith points out that a country should specialize in the production and exportation of goods that it can efficiently produce at a lower cost, *i.e.*, goods that provide it with an absolute advantage (Escobar, 2010). Therefore, a competitiveness analysis that identifies the strengths and weaknesses of the productive sectors of each country is fundamental. García *et al.* (2017) mention that competitiveness analysis identifies the comparative advantages of the different sectors of a country, as well as the factors that limit its growth and development.

Contreras and Leos (2021) mention that Bela Balassa (1965) proposed the first and most popular empirical measure of comparative advantage. Nevertheless, if a given crop is to be competitive, its comparative advantages should help producers to understand its benefits within the market.

The objective of this research was to carry out a competitiveness study to determine the comparative advantages of pecans and almonds within the international market and to identify their capacity to compete in each market. Data from the Sistema de Información Arancelaria Vía Internet (SIAVI) were used to carry out this study.

MATERIALS AND METHODS

The competitiveness of the Mexican pecans (*Carya illinoinensis*) K. Koch] and almonds (*Prunus dulcis*) and almonds in the international market was analyzed for the 2012-2021 period. The database was obtained from SIAVI and included exports and imports data for pecans, almonds, and all registered nuts from the study period.

The methodology used was the ECLAC (2008) Revealed Comparative Advantage Index (RCAi), which can be determined using the following formula:

$$RCAi = \frac{X_{ij} - M_{ij}}{X_{iw} + M_{iw}}$$

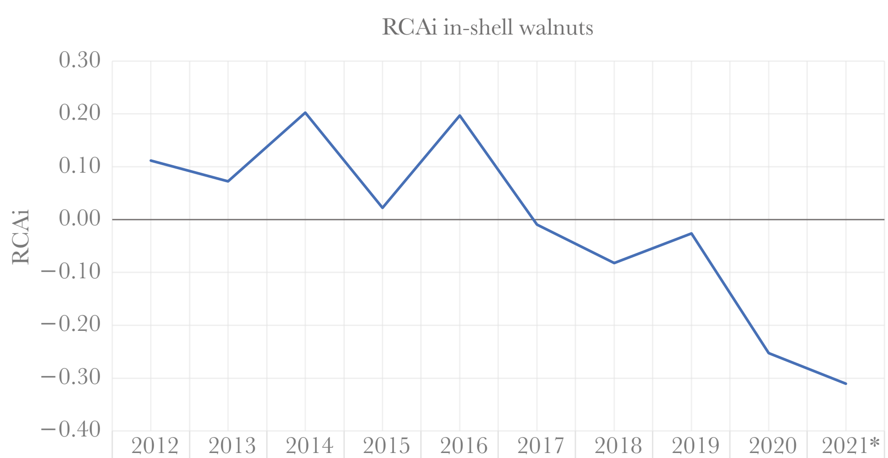
Where: $RCAi$ = Revealed Comparative Advantage Index; X_{ij} = Value of the Mexican product i exports in market j; M_{ij} = Value of the Mexican product i imports in market j; X_{iw} = Value of the Mexican product i total exports in the world market w; M_{iw} = Value of the Mexican product i total imports in the world market w.

According to ECLAC (2008), when RCAi is >1, the exports of pecans or almonds are higher than their participation in world trade (*i.e.*, they have comparative advantages); meanwhile, if the result is <1, the exports of pecans or almonds are lower than their participation in world trade (*i.e.*, they have no comparative advantages).

The purpose of the Revealed Comparative Advantage Index (RCAi) proposed by Balassa (1965) is to determine whether a country has any competitive advantage or not, depending on the differentiation of its international trade, during a given period and taking a given year as reference.

RESULTS AND DISCUSSION

In the 2012-2021 period, the world market demanded two types of nuts: in-shell and shelled walnuts. The latter are the most demanded nuts in the USA market. In 2016, the Revealed Comparative Advantage Index for in-shell walnuts recorded 0.20 —*i.e.*, walnut exports for that year were very competitive. In the following years, the RCAi decreased (Figure 1). These results indicate that there were no comparative advantages, because the RCAi was <1.



	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021*
RCAi	0.11	0.07	0.20	0.02	0.20	−0.01	−0.08	−0.03	−0.25	−0.31

Figure 1. RCAi (IVCR) of Mexico compared with the USA, HTS codes 0802.31.
Source: Developed by the authors with statistical data from SIAVI (2023).

Table 1. RCAi (IVCR) of in-shell walnuts, compared with the USA (2012-2021).

Year	EE. UU. $USD		World $USD		RCAi
	Exports	Imports	Exports	Imports	
2012	78,515,667	61,001,544	96,133,861	61,001,544	0.11
2013	64,586,072	54,727,103	81,331,587	54,727,103	0.07
2014	109,033,234	66,960,520	140,811,552	66,960,520	0.20
2015	99,434,497	94,164,229	146,009,609	94,164,229	0.02
2016	188,970,027	115,908,907	254,521,804	115,908,907	0.20
2017	113,450,584	115,916,125	143,114,621	115,916,125	−0.01
2018	155,168,382	185,325,370	183,402,241	185,325,370	−0.08
2019	131,993,689	141,733,878	236,327,561	141,733,878	−0.03
2020	81,325,543	151,283,796	125,823,966	151,283,796	−0.25
2021*	48,492,682	103,628,750	73,886,648	103,628,750	−0.31

* January-November.

Source: Developed by the authors with statistical data from SIAVI (2023).

Table 1 shows the RCAi of the in-shell walnut and its behavior and trend, during the analyzed period.

Comparing the results obtained from the analysis of the information from the SIAVI database with the research carried out by Ávila (2020) about the competitiveness and commercialization of the Mexican pecans, the demand for this dry fruit provides high benefits to the Mexican exports, particularly towards the USA, which is the most important commercial partner of Mexico.

Table 2 shows the RCAi of the shelled walnut and its behavior and trend, during in the analyzed period.

The Revealed Comparative Advantage Index (RCAi) for the shelled walnut was calculated to further the analysis of the studied period data and to evaluate the

Table 2. RCAi (IVCR) shelled walnuts, compared with the USA (2012-2021).

Year	EE. UU. $USD		World $USD		RCAi
	Exports	Imports	Exports	Imports	
2012	195,831,987	37,828,579	196,021,826	37,859,120	0.68
2013	177,965,218	18,865,523	178,088,097	18,865,523	0.81
2014	260,954,804	17,842,394	262,619,104	17,842,394	0.87
2015	299,424,991	18,930,793	301,046,505	19,118,203	0.88
2016	364,970,579	10,747,663	367,948,346	10,747,784	0.94
2017	441,210,238	15,419,897	444,566,207	15,431,369	0.93
2018	560,770,819	28,210,079	567,054,738	28,406,466	0.89
2019	557,257,978	29,592,621	567,916,720	30,130,266	0.88
2020	491,706,917	29,731,642	505,729,382	30,128,317	0.86
2021*	460,380,421	21,822,388	484,201,511	22,144,186	0.87

* January-November

Source: Developed by the authors with statistical data from SIAVI (2023).

competitiveness of this type of walnut in the USA market. Based on the results and the applied methodology, shelled walnuts have a ≈1 Revealed Comparative Advantage Index (Figure 2). This result (<1) indicates the existence of a Revealed Comparative Advantage in Mexico, regarding the shelled walnut exports to the USA.

Although the results identified that the Revealed Comparative Advantage Index follows a positive competitiveness trend, a higher value was recorded in 2016, followed by a decrease from that year on.

Ávila (2020) reviewed the results of the analysis of the competitiveness and marketing of the Mexican pecans in the international market and concluded that Mexico has a higher participation in the USA market. The behavior of Mexican exports has considerably increased both its competitiveness and its participation in the walnut market, keeping a stable trend over the years.

Nuts can also be sold in two different styles, each with its own HS code: whole or crushed/powdered. Whole nutmeg, mace, amoms, and cardamoms are included in the HS code 090811, while crushed or powdered nutmeg, mace, amoms, and cardamoms are included in the HS code 090812. Figure 3 shows the behavior of each fraction.

Whole nutmeg recorded a variable RCAi throughout the years. Table 3 show the variation in 2013, 2014, 2015, 2016, and 2017, showing a negative competitiveness. However, the RCAi improved and stabilized during the following years.

Figure 3 shows the variation of the Revealed Comparative Advantage Index and the lowest peak of competitiveness (2015). Thereafter, compared with the USA market, competitiveness increased and reached a RCAi of 0.07 in 2020, getting closer to 1.

Crushed or powdered nutmeg (HS code 090812) had a variable trend throughout the analyzed period, ranging from −0.21 (2012) to −0.12 (2021). In conclusion, there is no competitiveness for this type of nuts. In recent years, nutmeg has recovered its competitiveness; however, its RCAi is still negative.

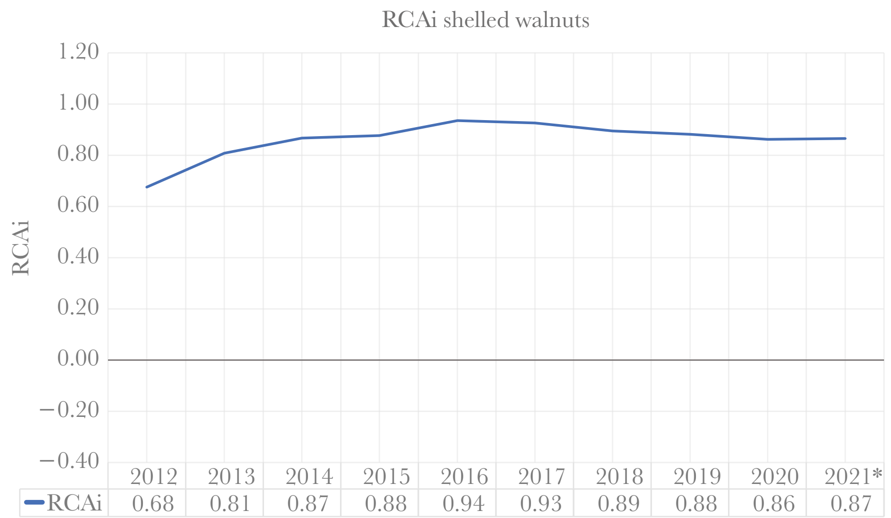


Figure 2. RCAi (IVCR) of Mexico, compared with the USA, Mexico HS code 08023201, shelled walnut. Source: Developed by the authors with statistical data from SIAVI (2023).

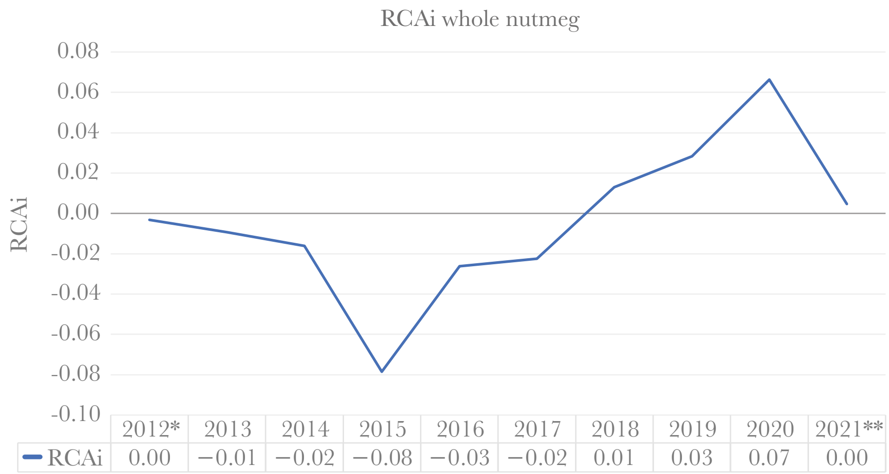


Figure 3. RCAi (IVCR) of Mexico, compared with the USA, HS code 090811, whole nutmeg, mace, amoms, and cardamoms. Source: Developed by the authors with statistical data from SIAVI (2023).

Table 3. RCAi (IVCR) of whole nutmeg, compared with the USA (2012-2021).

Year	EE. UU. $USD		World $USD		RCAi
	Exports	Imports	Exports	Imports	
2012*	10	515	1,835	151,542	0.00
2013	109	3,787	109	391,132	−0.01
2014	577	2,962	577	146,749	−0.02
2015	78	4,566	272	56,895	−0.08
2016	1,459	6,753	2,694	199,240	−0.03
2017	2,988	6,547	2,988	155,380	−0.02
2018	7,374	4,037	7,376	248,436	0.01
2019	6,957	5,221	6,957	54,301	0.03
2020	10,551	4,221	10,551	84,879	0.07
2021**	6,430	5,487	9,838	191,057	0.00

* July-December

** January-November

Source: Developed by the authors with statistical data from SIAVI (2023).

Likewise, export and import figures, as well as the Revealed Comparative Advantage Index, were used to measure the competitiveness of almonds in the USA market.

Table 4 shows the growth of the Mexican almond exports to the USA. Until November 2021, exports reached a very high level, while imports from the USA to Mexico also showed an increasing trend, reaching 69,762,997 units. These results shows that the RCAi was constantly below zero (−0.81 to −0.93); consequently, these data indicate that Mexico does not have a revealed comparative advantage regarding almond exports to the USA, regarding the world market.

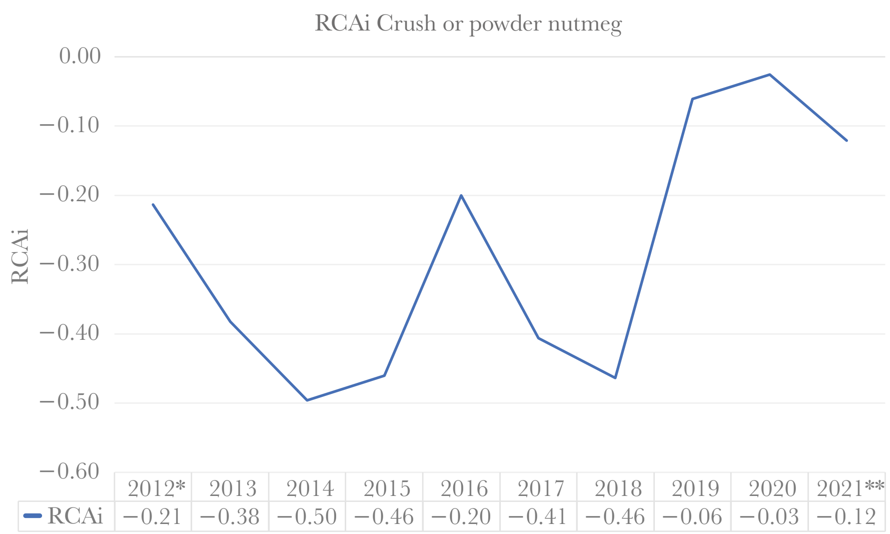


Figure 4. RCAi (IVCR) of Mexico, compared with the USA, HS 090812 Crush or powder nutmeg, mace, amoms, and cardamoms. Source: Developed by the authors with statistical data from SIAVI (2023).

Table 4. RCAi (IVCR) of Mexican shelled almonds, compared with the USA (2012-2021).

Year	EE. UU. $USD		World $USD		RCAi
	Exports	Imports	Exports	Imports	
2012	3,261,569	46,043,266	3,287,089	49,304,483	−0.81
2013	4,710,998	70,261,409	4,723,428	75,457,633	−0.82
2014	24,502	80,736,700	35,483	80,853,196	−1.00
2015	43	103,169,649	4,500,248	104,601,826	−0.95
2016	72,213	73,786,603	76,495	75,476,995	−0.98
2017	130,484	68,832,519	222,160	69,204,618	−0.99
2018	117,202	73,441,750	231,592	74,841,532	−0.98
2019	22,721	84,299,569	46,682	85,044,854	−0.99
2020	782,253	80,493,955	841,487	80,655,667	−0.98
2021*	2,164,339	69,762,997	2,169,749	70,584,759	−0.93

Source: Developed by the authors with statistical data from SIAVI (2023).

CONCLUSIONS

In a dynamic economic environment, competitiveness is a key element for the success and sustainability of both companies and countries. Competitiveness helps the ongoing adaptation, innovation, and improvement of commerce, maintaining and strengthening it in a globalized and highly competitive market. Throughout the years, the RCAi of Mexican pecans in the USA has remained consistently above zero (0.68-0.94). These results show that Mexico has a comparative advantage in pecan exports to the United States, compared with the world market. The results of the analysis show that Mexican pecans have a favorable competitive position in the USA market, as a result of its positive RCAi. Although Mexico has increased its almond exports to the USA, its competitiveness in both the USA market and the world market is still limited. The negative RCAi values indicate that other countries have higher efficiency and competitiveness regarding their almond exports. Overall, pecans are a competitive product in Mexico, as a result of their quality, demand, and the capacity of the producers to adapt to changing market conditions. Meanwhile, almonds are not a competitive product in Mexico. Although they are grown and produced in some regions of the country, the production and demand for almonds in Mexico is relatively lower than in the USA.

REFERENCES

Ávila Arce, A., González Milán, D. D. J., Montaño Méndez, I. E., & Vizcaíno Villareal, L. A. (2020). Análisis de la competitividad y comercialización de nuez pecanera mexicana en el mercado internacional. *Revista mexicana de ciencias agrícolas, 11*(4), 895-904.

Balassa, B. (1965). Trade Liberalisation and "Revealed" Comparative Advantage. The Manchester School. Volume 33, Issue 2. 99-123 pp

Bonilla Olano, E. V. (2012). La importancia de la productividad como componente de la competitividad.

Brambila Paz J.J. (2006). En el umbral de una agricultura nueva. Universidad Autónoma Chapingo.

CEPAL (2008). Indicadores de comercio exterior y política comercial: mediciones de posición y dinamismo comercial. José E. Durán Lima y Mariano Álvarez. Comisión Económica para América Latina y el Caribe. Naciones Unidas. Santiago de Chile

Contreras-Castillo, J.M. y Leos-Rodríguez, J.A. (2021). Nota metodológica sobre el índice de ventaja comparativa revelada normalizada. metodologías y herramientas para la investigación, V9. México: Universidad Autónoma Chapingo

Escobar, A. G. (2010). De la ventaja comparativa a la ventaja competitiva: una explicación al comercio internacional. Publicaciones Icesi.

García, F., Martínez, F., Díaz, H., & Molina, M. (2012). Evolución de la Ventaja Comparativa Revelada Normalizada en Productos Agropecuarios Mexicanos. 75-88

Mathews, J. (2009). Competitividad. El significado de la competitividad y oportunidades de internacionalización para las Mypes. 1a ed. Perú. Nathan Associates Inc.

Porter, M. (1980). Estratégia competitiva. Rio de Janeiro: Brasil. Editora Campus Ltda

Ramírez Díaz, L. F. (2006). La competitividad. ¿a qué se refiere? *Ensayos de Economía, 16*(29), 107–117. https://revistas.unal.edu.co/index.php/ede/article/view/24712

Ramírez Padrón, Laura Cecilia, Caamal Cauich, Ignacio, Pat Fernández, Verna Gricel, Martínez Luis, David, & Pérez Fernández, Alberto. (2020). Análisis de los indicadores de competitividad de las exportaciones de fresa mexicana. *Revista mexicana de ciencias agrícolas, 11*(4), 815-827. Epub 13 de septiembre de 2021. https://doi.org/10.29312/remexca.v11i4.2049

SIACON. (2023). Pecan Nut Production. Mexico.

SIAP. (2020). Anuario Estadístico de la Producción Agrícola. Servicio de Información Agroalimentaria y Pesquera.

SIAVI (2023). Estadísticas anuales. Sistema de Información Arancelaria Vía Internet. Consultado el 19 de mayo de 2023, disponible en línea en http://www.economia-snci.gob.mx/

Suñol, S. (2006). Aspectos teóricos de la competitividad. *Ciencia y Sociedad*, (2) Vol. 31 https://doi.org/10.22206/cys.2006.v31i2.pp179-198

Urrea López, R., & Urzúa Esteva, E. (2016). Challenges and Opportunities in Pecan Nut Production in Mexico. In N. D. Reyes Vázquez, & R. Urrea López, Challenges and Opportunities for the use of the Pecan Nut in Mexico (p. 2).

Field identification of Huanglongbing (HLB) and its management alternatives

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ABSTRACT

Objective: The objective of this investigation is to identify huanglongbing (HLB) in the field and know their control altenatives.

Design/methodology/approach: Was investigated the introduction of the phytopathogen *Candidatus* Liberibacter, symptoms and control of HLB. The generally observed symptoms of this disease are irregular yellowing in the leaves and reversed ripening on the fruit.

Results: According to various authors, an integrated control of the disease must be carried out, such as pest control, production of seedlings in certified nurseries, a suitable nutrition management and working together with educational and research institutions.

Limitations on study/implications: HLB or citrus greening is the most devastating disease of citrus trees which has caused millions of dollars in losses worldwide. However, although there are several research, there is still no established cure.

Findings/conclusions: Research such as that carried out by geneticist Hailing Jin at the University of California Riverside can give hope for the cure or control of this disease, however, there are limitations to this product since it is not yet commercialized.

Keywords: Identification, Huanglongbing, control, citrus crops.

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INTRODUCTION

Citrus farming is an agricultural and economic activity of great global importance, (Liu *et al.*, 2012), since citrus fruits are produced in more than 140 countries, mainly in China, Brazil, India, and Mexico (FAOSTAT, 2021). However, one of the limitations to production is the damage caused by diseases, particularly Huanglongbing (HLB), commonly known as citrus greening (Sáenz-Pérez *et al.*, 2019). HLB is the most devastating disease of citrus trees worldwide since it reduces the production and quality of the fruit, resulting in economic losses (Bove, 2006), which vary according to the crop. For example, losses of up to 42, 62, and 17.3% have been reported in oranges, Mexican lime, and Persian lime, respectively (Mora-Aguilera *et al.*, 2014). In 2013, HLB was present in 39 countries; however, by 2019, it was reported in 71 countries, including the main citrus producers (CABI, 2019; EPPO, 2019). There are different types of *Candidatus* Liberibacter, depending on the country: *Candidatus*

Liberibacter asiaticus is present in Asia, Africa, Oceania, and the Americas, (CABI, 2019); *Candidatus* Liberibacter africanus can be found in Asia and Africa; *Candidatus* Liberibacter americanus has been reported in the Americas (EPPO, 2019); and *Candidatus* Liberibacter caribbeanus is found in Colombia (Keremane *et al.*, 2015).

MATERIALS AND METHODS

First, literature about HLB disease was reviewed, placing special emphasis on its symptoms and management alternatives. Subsequently, a visual monitoring of citrus orchards was carried out in Tamaulipas, identifying symptoms, and taking photographs in the field.

RESULTS AND DISCUSSION

How the HLB infects the tree

HLB is caused by the *Candidatus* Liberibacter spp. bacteria and is mainly transmitted by the *Diaphorina citri* vector (Zhang *et al.*, 2014). *Candidatus* Liberibacter spp. can be found in the hemolymph and salivary glands of the insect vector and it enters the citrus tree through the stylet of the psyllid when the vector sucks the sap. It then moves through the sieve tubes of the phloem, until it reaches the root, where it replicates and subsequently spreads to the rest of the tree (Johnson *et al.*, 2013).

HLB symptoms

The characteristic symptoms of HLB in citrus are the yellow, asymmetrical mottling of the leaves and growth retardation in infected plants. HLB can be identified through sporadic spots in the leaves and the lack of a similar pattern when both leaf blades are folded (Figure 1). Nevertheless, it can be confused with nutritional deficiencies; therefore, a proper identification is required (Zhi *et al.*, 2021). The leaves may also show a corky appearance and dilated veins (Bove, 2006).

Fruits infected with HLB may be smaller and asymmetrical and show inverse ripening (Figure 2), thickening of the pericarp (peel), and seed abortion, which decreases fruit quality and affects its flavor. The reduction of its organoleptic quality nullifies fruit trade in the industry (Bove, 2006). This disease also causes premature leaf drop, fruit abortion, and, in advanced stages, the death of the citrus tree (Wang *et al.*, 2017).

Figure 1. HLB symptoms in leaves. The irregular yellowing changes as the disease progresses.

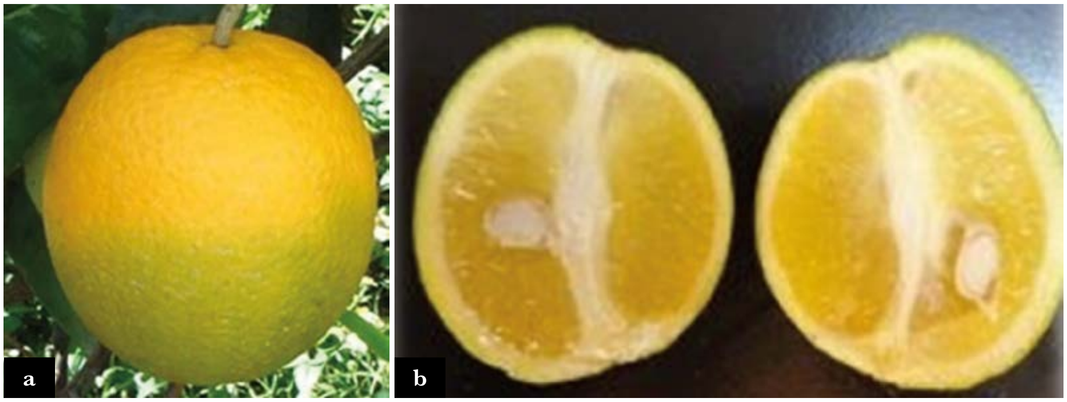

Figure 2. HLB symptoms in fruit: a) inverse ripening and b) asymmetry.

HLB management alternatives

There are various alternatives for the management and prevention of HLB, but there is still no cure for trees infected with this disease (Gottwald *et al.*, 2007).

Vector management

Although *Diaphorina citri* is the main vector of the *Candidatus* Liberibacter bacterium (Hall, 2018), it can also be spread by *Trioza erytreae* and other vectors (Zhang *et al.*, 2014). Since this bacterium is found in the salivary glands of the insect, it enters the tree through the stylet of the psyllid when the vector sucks the sap (Johnson *et al.*, 2013). Therefore, managing these vectors is the main strategy for HLB management (Ruiz-Galván *et al.*, 2015).

The following types of vector management are available:

1. Cultural management: providing adequate nutrition to make the plant resistant to pathogens and pests (Velasco, 1999), detecting insects before they cause damage, and promptly identifying infected plants (Mann *et al.*, 2018).
2. Biological management: using natural predators of *Diaphorina citri*, including the *Cycloneda sanguinea*, *Chilocorus cacti*, *Exochomus cubensis*, *Scymnus distinctus*, *Chrysopa* sp., and *Ocyptamus* sp. ladybugs (Miranda *et al.*, 2008), or parasitoids wasps, both ectoparasitoids (*e.g.*, *Tamarixia radiata*) and endoparasitoids (*e.g.*, *Diaphorencyrtus aligarhensis*) (Grafton-Cardwell *et al.*, 2013). Entomopathogenic fungi capable of infecting the insect vector and subsequently causing its death can also be used, including *Isaria fumosorosea* and *Beauveria bassiana* (Saldarriaga *et al.*, 2017).
3. Chemical management: the most popular control method among producers (Tiwari *et al.*, 2011). Neverthless, these products should only be applied before the maximum population peaks, which are usually recorded at the beginning of the year, when favorable conditions for the population increase of the vector are reported (Cortez *et al.*, 2010). The most common insecticides are temik, imidacloprid, dimethoate, chlorpyrifos, cypermethrin, malathion, deltamethrin, β-cyfluthrin, spirotetramat, spinetoram, oxamyl, tricarboxylic acids, omethoate, sulfoxaflor, and mineral oils, among others (Orozco and Cano, 2012).

Use of antibiotics

In Florida, United States, streptomycin sulfate, oxytetracycline hydrochloride, and oxytetracycline calcium complex have been approved as treatments against HLB. These foliar antibiotics are applied as aerosols. Nevertheless, no studies have proven the benefit of these compounds (Wang *et al.*, 2017).

Thermotherapy management

Thermotherapy seems to be an effective method for HLB management, at least in greenhouse and growth chambers. Fan *et al.* (2016) exposed mandarin orange seedlings infected with HLB to a temperature of 45-48 °C for 4 h, 1 day a week, for 3 weeks in a row. As a result, the phytopathogen decreased by 30 to 55% and the control increased by 300%. However, the effectiveness of thermotherapy depends on both the components of the plant and the soil. This is an expensive and slow alternative in the field, although equipment is being developed to make it feasible on a larger scale (Trotochaud and Ehsani, 2016).

Alternative management

Scientists at the University of California, Riverside, found a peptide that can control citrus greening. Geneticist Hailing Jin determined that this peptide is stable outdoors, withstands high temperatures, is safe for humans, and reduces the pathogen to a considerable degree; however, it is not yet commercialized, and more research is required before it can be used (Bernstein, 2020).

Management with the addition of nutrients

In agriculture, fertilization covers the nutritional requirements of the plants, providing quality to the production. Likewise, it can also be used to manage diseases (Huber, 1989; Fageria *et al.*, 1997); however, a nutritional balance must be first carried out, to establish the resistance of the plant to different pathogens (Velasco, 1999), since nutritional excess or deficiencies have been proven to be able to modify susceptibility to diseases (Velasco, 1999; Huber, 1978). According to Giles (2011), macronutrient and micronutrient foliar sprays increased the vigor and productivity of citrus trees infected with HLB. Despite their high quantities, the nutrients in the soil do not meet the requirements of the crop, since some elements cannot be assimilated by the plant; this deficiency can only be compensated by the application of fertilizers (Zúñiga, 2013). Plants require at least 17 essential elements for their development, growth, and metabolism, including O, H, and C, which they obtain from H_2O, CO_2, and air. The rest are mineral nutrients, which are classified as micronutrients or macronutrients, according to the amount absorbed. Macronutrients are found in higher concentration in plant tissue; they are divided into primary elements, such as N (nitrogen), P (phosphorus), and K (potassium) and secondary elements, such as Ca (calcium), Mg (magnesium), and S (sulfur). The micronutrients are B (boron), Cl (chlorine), Cu (copper), Fe (iron), Mn (manganese), Mo (molybdenum), Ni (nickel), and Zn (zinc) and are required in lower concentrations (Marschner, 2011).

Strategies for HLB management

Given the lack of a cure for this disease, an integrated management must be implemented to reduce the symptoms and the death of trees infected with HLB. The said management consists of the following measures: determining how this disease enters the plant, in order to control the vector (Zhang *et al.*, 2014); establishing the temperatures at which the pathogen decreases (Fan *et al.*, 2016); finding out when the plant should be nourished to generate resistance (Velasco, 1999); and determining what product can be applied before and during the disease (Wang *et al.*, 2017).

International strategies for HLB management

The Food and Agriculture Organization of the United Nations (FAO) and the United Nations Development Program (UNDP) developed strategies that were accepted as an international management for HLB (Aubert, 1990). This program consists of an integrated management: the control of psyllids with insecticide, the elimination of trees with HLB symptons (to reduce the inoculum), geographical isolations, and nursery certifications (to propagate pathogen-free trees). To improve disease management, citrus phytosanitary agencies in Florida and Brazil require that tree production be carried out under insect-proof greenhouses, to enhance the management of the insect vector (Bassanezi *et al.*, 2011).

National HLB management strategies

In Mexico, the strategies aimed at controlling HLB consist of a set of phytosanitary activities (Da-Graca and Kortsen, 2004) that involve producers, auxiliary plant health agencies, educational institutions, and research institutions (SENASICA, 2010). The tripartite strategies take place within the context of epidemiological surveillance. The first measure is the NOM-EM-047-FITO-2009 standard —which establishes phytosanitary actions to mitigate the risk of introduction and spread of HLB—; the second is eradication —which determines how to control the disease and the psyllid—; and the third is chemical and/or biological management (Mora-Aguilera, 2013).

CONCLUSIONS

Given the current lack of established management for HLB, preventive measures must be taken, beginning using seedlings from certified nurseries, followed by soil and plant analysis that will help to determine an adequate and balanced nutrition and ultimately to generate resistance against this pathogen. The insect vector will not be able to generate resistance, if cultural, biological, and chemical management measures are applied in this precise order and there is no excessive use of chemicals.

REFERENCES

Liu, Y., Heying, E. y Tanumihardjo, SA .2012. Historia, distribución mundial e importancia nutricional de los cítricos. *Reseñas completas en Food Science and Food safety, 11*(6), 530-545.

FAOSTAT (Food Agriculture Organization Data). 2021. Datos sobre alimentación y agricultura. En: http://www.fao.org/faostat/en#data.

Sáenz-Pérez, C. A., Hernández, E. O., Estrada-Drouaillet, B., Poot-Poot, W. A., Delgado-Martínez, R., y R. R., Herrera. 2019. Principales enfermedades en cítricos. *Revista mexicana de ciencias agrícolas* 10: 1653-1665.

Bove, J. M. 2006. Huanglongbing: a destructive, newly-emerging century-old disease of citrus. *Journal Plant Pathology* 88: 7-37.

Mora-Aguilera, G. 2013. Huanglongbing Candidatus liberibacter spp. Ficha técnica 78. En: SADER y SENASICA(Ed.). *Vigilancia Epidemiológica Fitosanitaria*. pp 3-28.

CABI (Centro de Biociencia Agrícola Internacional). 2019. Distribution Maps of Plant Disease. *Candidatus* Liberibacter asiaticus. Distribution map. In: https://www.cabi.org/dmpd/abstract/20 173134805.

EPPO (European Plant Protection Organization). 2019. Global database.in: https://gd.eppo.int/search?k=citrus+.

Zhang, M.; Guo, Y.; Powell, C. H. A.; Doud, M. S.; Yang, C. H. and Duan, Y. 2014. Effective antibiotics against '*Candidatus* Liberibacter asiaticus' in HLB affected citrus plants identified via the graft-based evaluation. *PLOS ONE. 9*(11):1-11.

Johnson, E. G.; Wu, J.; Bright, D. B. and Graham, J. H. 2013. Root loss on presymptomatic huanglongbing affected trees is preceded by *Candidatus* Liberibacter asiaticus root infection but not phloem plugging. *Plant Pathol. 63*(2):290-298.

Keremane, M., Ramadugu, L. C., Castaneda, A., Diaz, J. E., Peñaranda, E. A., Chen, J., Duan, Y. P., Halbert, S. E. and R. F., Lee. 2015. Reports of *Candidatus* Liberibacter caribbeanus, a new citrus and psyllid associated Liberibacter from Colombia South America. APS Annual Meeting August. 1-5 p.

Zhi, H. D., Anoop, K. S., Xiao, D. L., Muhammad, R., Yu, G., Xiao, M. L., Qi, L.T.T., Xue, C. S., Song, W. W. and X.H., Cheng. 2021.Interactions between nutrient and Huanglongbing pathogen in citrus. *Scientia Horticulturae* 290: 304-4238.

Wang, N., Pierson, E.A., Setubal, J.C., Xu, J., Levy, J.G., Zhang, Y., Jinyun, L., Rangel, L.T., and J. J., Martins. 2017. The *Candidatus* Liberibacter host interface: insights into pathogenesis mechanisms and disease control. *Annual Review of Phytopathology* 55: 451-482.

Gottwald, T. R.; Da-Graca, J. V.; Bassanezi, R. B. 2007. Citrus Huanglongbing: The pathogen and its impact. Online. *Plant Health Progress.*

Hall, D. G. 2018. Incidence of *Candidatus* Liberibacter asiaticus in a Florida population of Asian citrus psyllid. *Journal of Applied Entomology 142*(1):97-103.

Zhang, M.; Guo, Y.; Powell, C. H. A.; Doud, M. S.; Yang, C. H. and Duan, Y. 2014. Effective antibiotics against '*Candidatus* Liberibacter asiaticus' in hlb-affected citrus plants identified via the graft-based evaluation. *PLOS ONE. 9*(11):1-11.

Ruiz-Galván, I., Bautista-Martínez, N., Sánchez-Arroyo, H., & Valenzuela Escoboza, F. A. 2015. Control químico de *Diaphorina citri* (Kuwayama)(Hemiptera: Liviidae) en lima persa. *Acta zoológica mexicana, 31*(1), 41-47.

Velasco, V. A. V. 1999. Papel de la nutrición mineral en la tolerancia a las enfermedades de las plantas. *Terra Latinoamericana* 17: 193-200.

Grafton-Cardwell, E.E., Stelinski, L.L. and Stansly, P.A. 2013. Biology and management of Asian citrus psyllid, vector of the huanglongbing pathogens. *Annual Review of Entomology*. 58: 413-432.

Saldarriaga Ausique, J.J., D'Alessandro, C.P. and M. R. Conceschi. 2017. Efficacy of entomopathogenic fungi against adult *Diaphorina citri* from laboratory to field applications. *Journal Pest Science* 90, 947–960.

Tiwari, S., Mann, R. S., Rogers, M. E. and Stelinski, L. L. 2011. Insecticide resistance in field populations of Asian citrus psyllid in Florida. *Pest Management Science*, 67: 1258-1268.

Cortez, M. E., López A., J. I., Hernández, L. M., Fú, A. and Loera, J. 2010. Control químico de *Diaphorina citri* Kuwayama en cítricos dulces, en México. Selección de insecticidas y época de aplicación. Folleto técnico No. 35 INIFAP-SAGARPA.obla

Orozco, S. M. y Cano, H. A. 2012. Evaluación de la efectividad biológica del insecticida imidacloprid + bifenthrin 250/50 para el control del Psílido Asiático (*Diaphorina citri*) en el cultivo de limón mexicano (*Citrus aurantifolia*), en Tecomán, Colima. 2 Simposio nacional sobre el control del Psílido Asiático de los cítricos y el Huanglongbing.

Fan, G.C., Xia, Y.L., Lin, X.J., Hu, H.Q., Wang, X.D., Ruan, C.Q., Lu, L.M. y Liu, B. 2016 . Evaluación de la termoterapia contra el huanglongbing (enverdecimiento de los cítricos) en invernadero. *J.Integr. agricola* 15:111-119.

Trotochaud, J. y Ehsani, R. 2016. Termoterapia a escala comercial para combatir el enverdecimiento de los cítricos (huanglongbing). Internacional ASABE Reunión.

Huber, D. M. 1989. Introduction. In: A.W. Engelhard (Ed.). Soilborne plant pathogen: management of disease with macro and microelements. APS Press, United State of America. pp 1-8.

Fageria, N. K., Baligar, V. C. y Ch. A., Jones. 1997. Growth and mineral nutrition of fields crops. Marcel Dekker (Ed.). United State of America. pp 1: 1-11.

Huber, D. M. 1978. Disturbed mineral nutrition. In: J.G. Horsfall y E.B. Cowling (Eds.). Plant disease and advanced treatise. Vol. 3. Academic Press, United State of America. pp 163-181.

Giles, F. 2011. Daring to be different. Growing produce. United State of America.1-4 p.

Zuñiga, L., E. 2013. Nutrición de Agave tequila y manejo de los fertilizantes en un sistema de producción intensivo (riego por goteo). INIFAP (Instituto Nacional de Investigación Forestales, Agrícolas y Pecuarias), México.

Marschner, H. 2011. Marschner's mineral nutrition of higher plants. San Diego, CA, USA: Academic Press.

Marschner, P. 2012. Mineral nutrition of higher plants, 3rd edn. Elsevier, Oxford.

Aubert, B., 1990. Integrated activities for the control of huanglungbin-greening and its vector *Diaphorina citri* Kuwayama in Asia. In: Aubert, B., Tontyaporn, S., Buangsuwon, D. (Eds.), Rehabilitation of Citrus Industry in the Asia Pacific Region. Rome: UNDP-FAO. pp 133-144.

Bassanezi, R. B., Yamamoto, P. T., Montesino, L. H., Gottwald, T. R., Amorim, L., and F. A., Bergamin. 2011. Regional HLB Management on the Effectiveness of Local Strategies of Inoculum Reduction and Vector Control. *Plant Disease*. pp 176.

Da-Graca, J. V. and L., Korsten. 2004. Citrus Huanglongbing: Review, Present status and Future Strategies. *Diseases of fruit and Vegetables* 1: 229-245.

SENASICA (Servicio Nacional de Sanidad, Inocuidad y Calidad Agroalimentaria). 2010. Estrategias en México contra el HLB. En: http://www.senasica.gob.mx.

Bernstein J. 2020. UC Riverside descubre el primer tratamiento eficaz para la enfermedad que destruye los citricos, nuevo acuerdo de licencia comercializa tecnologia innovadora y segura. UC Riverside noticias. In: https://news.ucr.edu/articles/2020/07/07/uc-riverside-discovers-first-effective-treatment-citrus-destroying-disease.

Phenotypic plasticity and biomass allocation in fertilized *Quercus variabilis* Blume seedlings

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ABSTRACT

Objective: To determine the phenotypic plasticity between fertilized (F) and unfertilized (UF) *Q. variabilis* seedlings developed in a nursery using small, medium, and large seeds.

Design/methodology/approach: By analyzing the phenotypic traits of growth (height and diameter at root collar) and root and stem dry biomass, plasticity indices and allocation patterns were assessed, and phenotypic traits in which the interaction effect of the seedling fertilization and seed size was observed.

Results: The results showed significant differences (p-value≤0.05) in the growth and biomass traits except for the root dry biomass, and significant differences were found both in the fertilization and seed size factors and in the fertilization and seed size interaction in phenotypic traits. For plasticity, all traits showed marked changes in response to the nutrient application, and small seeds had the highest plasticity indices. With respect to phenotypic change indices, medium seeds reached the highest values (0.94 for height and 0.92 for diameter). Large seeds recorded the highest index for root dry biomass (1.01), and medium seeds had the highest values of plasticity for stem dry biomass (0.81). Allometric differences were observed (intercepts were 1.8374 and 3.4956, and slopes were 4.0943 and 1.7038 for UF and F seedlings, respectively), and variations in the study factors (fertilization and seed size).

Limitations on study/implications: In order to improve the survival of plants in the field it is necessary to use seeds with high quality.

Findings/conclusions: Fertilization of *Q. variabilis* seedlings increases their biomass production, which allows for greater growth and survival compared to unfertilized ones.

Keywords: Environmental variation, Forest conservation, Phenotypic variation, Seed size.

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INTRODUCTION

Quercus genus is important in the temperate and tropical forests of the northern hemisphere. It comprises approximately 450 species, with differences in their flowering, fruiting, and maturation, due to their phylogenetics and ecological factors present in their distribution (Vinha *et al.*, 2016). In China, there are 40 oak species, which stand out for their role in maintaining biodiversity and the stability of the ecosystems they form (Sun *et al.*, 2020). In this country, the study of its taxa is a current issue for conservation in some of oak species, *e.g.*, *Quercus variabilis* Blume, *Q. mongolica* Fisch. ex Ledeb., *Q. acutissima* Carruth.

Quercus variabilis has high ecological, economic, and cultural value in China (Gao *et al.*, 2018). To conserve this resource in the face of the constant degradation of the forests it harbors, some research has been carried out, *i.e.*, the current status and potential distribution under climate change scenarios (Sun *et al.*, 2020), factors affecting seedling regeneration in different climatic regions (Wu *et al.*, 2013), inter-and intraspecific phenotypic variation (Feng *et al.*, 2021), and population structure and dynamics (Zhang *et al.*, 2008). These topics were part of the global strategy for plant conservation in China (2011-2020), but further research is still required to maintain and rehabilitate the current population of the species.

In plants, nutrients not only play an important role in tissue development but also help them to adapt to environmental variations. This has highlighted the importance of fertilization for nutrient supply given the continuing effects of climate change. For example, global warming can reduce nutrient reabsorption, while drought can accelerate leaf senescence and increase reabsorption (Rivero *et al.*, 2007). Thermal changes brought about by global warming affect the distribution and abundance of plants, in addition to their respiration, and limit the availability of minerals in the soil that can reduce their growth responses and biomass production. Increased atmospheric humidity leads to change in nutrient storage, growth, and development, which can be reversed by supplying macronutrients (Oksanen *et al.*, 2018). Studying the adaptive potential of forest species and their response to stimuli such as fertilization is a priority for their continuity and to achieve greater survival and growth at their planting sites.

Currently, the drought tolerance evaluation of forest species to climate change scenario is increasingly relevant. In this regard, using available data from online repositories to analyze species responses is a valid method (Mendeley, Zenodo), given that these are databases considered to have the potential to assess their plastic responses in different climatic gradients (Vizcaíno-Palomar *et al.*, 2019).

Studying a species' phenotypic plasticity (the capacity to alter phenotype in direct response to environmental changes (Costa, 2021)) allows to understand and predict its response to changes in the environmental factors in which it is found, and the performance of the environmental and genetic components (Pazzaglia *et al.*, 2021). Seed size is an important factor for analyzing phenotypic plasticity since it is a variable that evolves in responding to different environmental stresses (Li *et al.*, 2021). There is limited research available for *Q. variabilis* on plasticity in the last decade, based on different seed sizes (Xu *et al.*, 2015), which makes it difficult to understand how the species will adapt to different scenarios.

Therefore, the objective of this research was to determine the phenotypic plasticity of fertilized and unfertilized *Q. variabilis* seedlings produced in a nursery from small, medium, and large seeds.

MATERIALS Y METHODS

Plant material data

Data collected on fertilized and unfertilized seedlings were obtained from the repository (greenhouse experiment) and used in this study for analyze the *Q. variabilis* plasticity. At present, databases from online repositories is considered to have the potential to assess plastic responses of plants. For consultation, this information is available at the Mendeley repository (https://doi.org/10.17632/krgxdd2rtp.2). From this database, four variables were selected: two phenotypic growth traits (height (cm), and root collar diameter (cm)) and two biomass traits (root dry biomass (g), and stem dry biomass (g)). These variables were chosen because they have been used to evaluate the plasticity, and their variations under different environments.

The greenhouse experiment was undertaken during 2014 to test the impact of fertilization in seedling morphology and nutrition in the nursery and outplanting performance (Shi *et al.*, 2019). The trial was established in the Beijing Forestry University near Jiufeng Mountain, Beijing having 28.5 °C day and 16.5 °C night temperature and 84.7% relative humidity. Seedlings were produced from three seeds of different sizes: small (2.88 ± 0.09 g), medium (4.18 ± 0.10 g), and large (5.52 ± 0.27 g), which were defined using fresh weight (g) and by performing cluster analysis. Four hundred seeds ofeach size class were sown in cylindrical, hard plastic D60 containers: one seed per container (1-2 cm of depth). The container diameter was 6.4 cm and the depth was 36.0 cm, resulting in a volume of 983 ml. The growing medium was a 3:1 (v:v) mixture of peat (pH 6.0, screening 0-6mm) and perlite (5 mm diameter).Seventy-five seedlings (25 individuals of each seed size) were fertilized with 20 ml 20N-20P-20K solution for 10 weeks while 75 (25 individuals of each seed size) were not. After this growing period, each fertilized plant ($n=75$) was supplied with 100 mg of N. The experiment was established in a nursery, for 195 days, following a completely randomized design.

Plasticity and phenotypic change indices

Based on different seed sizes, plasticity indices (PI) were determined for phenotypic traits of seedlings when the fertilization factor was significant at 0.05, *i.e.*, fertilized seedlings. Plasticity indices were calculated following the Hernández-Pérez *et al.* (2001) equation to assess the association degree between the species' response to fertilization and its growth potential.

The indices of phenotypic change were determined based on the graphical method proposed by Pigliucci and Schlichting (1996), in which the average values of the phenotypic traits of seeds (of different sizes) are represented in a two-dimensional plane. The abscissa axis corresponds to unfertilized seedlings, while the ordinate axis corresponds to fertilizedseedlings.

Statistical analysis

Firstly, Mardia's multivariate normality test (H_0: The variables come from a multivariate normal distribution) (Mardia, 1974) was applied to the height, root collar diameter, root dry biomass, and stem dry biomass variables. As the statistic value was 36.0903 and the p-value was 0.0150, *i.e.*, less than the significance level (p-value<0.05), it was concluded that the data did not come from a multivariate normal distribution. Therefore, statistical analyses using non-parametric statistics was perform, specifically to determine the phenotypic traits that had a significant response to fertilizer addition. Mann-Whitney-Wilcoxon test and non-parametric multivariate analysis of variance were applied to determine the factors (Fertilization, seed size, and interaction) which affected the plant growth. All analyses were performed with R statistical software version 3.2.3. (R Core Team, 2015).

To assess the biomass allocation patterns, an allometric analysis of the changes between root dry biomass and stem dry biomass was performed. For this purpose, a regression model with indicator variables was used to determine whether the behavior of root dry biomass differed in both the intercept and slope as a function of stem dry biomass and fertilization. To obtain the test statistic and conclude based on the stated hypothesis, the extra sum of squares method was applied (Montgomery *et al.*, 2012).

To complement the interpretation of the results from the experimental design with a multivariate approach, a Canonical Discriminant Analysis was performed, which allowed to identify differences between groups (treatments), based on the characteristics (variables) measured on the individuals of these groups, and understand the relation between the variables within the groups. The latter was defined by the combinations between fertilization levels (fertilized and unfertilized seedlings) and seed size (small, medium, and large). With the Canonical Discriminant Analysis, canonical variables were obtained from the original variables (phenotypic traits), with which the separation and conformation of groups of individuals were achieved by maximizing the variance between groups and minimizing the variance within groups. Finally, Mahalanobis distances were calculated in order to know the groups.

RESULTS AND DISCUSION

The results show that the application of fertilizer on *Q. variabilis* seedlings produced from different seed sizes, promoted phenotypic and plasticity changes, as well as differences in their allocation patterns. This is because the species respond differently during growth and biomass production (stem and root) to the fertilization stimulus imposed (Agathokleous *et al.*, 2022; Pająk *et al.*, 2022), at an early age and independent of the germplasm size.

Fertilization in *Q. variabilis* seedlings significantly affected their morphological traits, according to the Mann-Whitney-Wilcoxon (W) test (Table 1), *i.e.*, the means of all morphological traits showed a significant difference (0.05) between fertilized and unfertilized seedlings, except for the root dry biomass trait. With regard to the non-parametric multivariate analysis, significant differences were found both in the fertilization (p-value=0.0001) and seed size (p-value=0.0001) factors and in the fertilization and seed size interaction (p-value=0.0001) in phenotypic traits height, root collar diameter, root dry biomass, and stem dry biomass. Fertilized seedlings showed higher growth, thus higher biomass production than unfertilized seedlings. Seedlings germinated from large and medium seeds obtained the highest values in height, root collar diameter, root dry biomass, and stem dry biomass compared to seedlings produced from the smaller seeds (Table 2).

Table 1. Averages of morphological attributes of *Quercus variabilis* seedlings.

Trait†	**Test statistics (W)**	**p-value**	**Level**‡	
			F	**UF**
H	1846.5	0.0002	31.7[a]	27.8[b]
D	1744.0	5.95×10^{-05}	3.5[a]	3.2[b]
RDB	2319.0	0.06379	5.1[a]	4.7[a]
SDB	1520.0	1.19×10^{-06}	0.9[a]	0.7[b]

†H: Height (cm), D: Root collar diameter (mm), RDB: Root dry biomass (g·seedling^{-1}), SDB: Stem dry biomass (g·seedling^{-1}). ‡F: Fertilized seedlings, UF: Unfertilized seedlings. Different letters in each line indicate significant differences (α=0.05) due to fertilization.

Table 2. Growth and biomass of *Q. variabilis* seedlings by seed size. Based on Student's t-test, different letters per column indicate significant differences (p-value=0.05) due to fertilization.

Seed size	**Level**†	**H**‡	**D**	**RDB**	**SDB**
Large	F	36.59[a]	3.80[a]	5.27[a]	1.23[a]
Large	UF	30.53[b]	3.51[b]	5.34[a]	0.89[b]
Medium	F	32.72[a]	3.56[a]	5.34[a]	0.96[a]
Medium	UF	30.74[a]	3.29[b]	5.19[a]	0.78[b]
Small	F	25.66[a]	3.22[a]	4.69[a]	0.64[a]
Small	UF	22.20[b]	2.86[b]	3.66[b]	0.45[b]

†F: Fertilized plant, UF: Unfertilized plant. ‡H: Height (cm), D: Root collar diameter (mm), RDB: Root dry biomass (g·plántula^{-1}), SDB: Stem dry biomass (g·seedling^{-1}).

The interaction between seed size and fertilization of *Q. variabilis* seedlings affected the growth and biomass variables. This variation occurred because fertilized seedlings tend to increase in size on providing nutrients (Dziedek *et al.*, 2017). Similar results in the growth have been reported in *Quercus suber* L. (Mechergui *et al.*, 2021), while biomass has been reported for *Quercus brantii* Lindl (Yadegari and Seyedi, 2019); both aspects are related to the amount of protein, carbohydrate, and lipid reserves because these promote the growth and biomass production (Mechergui *et al.*, 2021). It has been reported that genetic component plays an important role in the interaction between seed size and fertilization (Clark and Schlarbaum, 2018), which could have been the reason for observing differences in the interrelationship between these two factors.

Q. variabilis seedlings showed marked changes (plasticity) in all traits in response to the nutrient application, which highlighted the importance of fertilization in this species (Table 3). For most traits, small seeds showed the highest plasticity indices, while medium and large seeds showed the least (Table 3).

The changes in phenotypic indices three seed sizes were not distinct but showed changes in the analyzed traits (Figure 1). For the growth variables, medium seeds reached the highest values (0.94 for height and 0.92 for root collar diameter), while small seeds had the lowest values (0.86 for height and 0.88 for root collar diameter) (Figure 1a, b). Regarding

Table 3. Phenotypic plasticity indices of *Quercus variabilis* seedlings germinated from seeds of different sizes.

Trait†	Index		
	Small seed	Medium seed	Large seed
H	13.50	6.03	16.56
D	11.04	7.53	7.66
RDB	21.95	2.92	−1.44
SDB	29.78	18.56	27.36

†H: Height (cm), D: Root collar diameter (mm), RDB: Root dry biomass (g·seedling^{-1}), SDB: Stem dry biomass (g·seedling^{-1}).

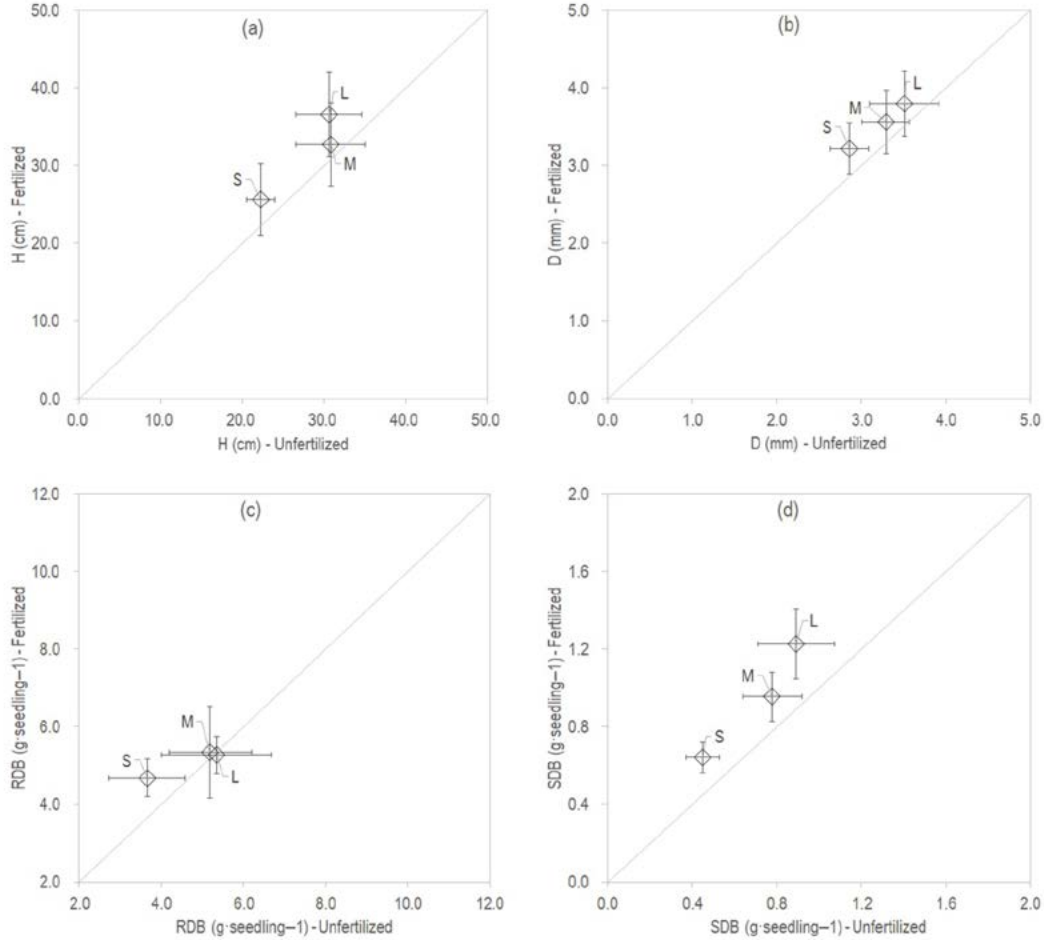

Figure 1. Plasticity analysis by seed size (S: small, M: medium and L: large) for growth variables and biomass components with a significant response to fertilization treatment in (a) H: Height (cm), D: Root collar diameter (mm), RDB: Root dry biomass, SDB: Stem dry biomass. The bars indicate the standard errors in the two treatments.

biomass components, large seeds recorded the highest index for root dry biomass (1.01) (Figure 1c), while medium seeds had the highest values of plasticity for stem dry biomass (0.81) (Figure 1d).

For small seeds, growth plasticity indices showed higher values for diameter; however, in biomass-related plasticity indices higher values were obtained for all traits, although larger seeds showed higher values for height. These results varied from those reported in ten plant species (*Populus tremuloides* Michx., *Betula papyrifera* Marsh, *Betula alleghaniensis* Britton, *Acer saccharum* Marsh, *Larix laricina* (Du Roi) K. Koch, *Pinus banksiana* Lamb., *Pinus resinosa* Ait., *Pinus strobus* L., *Picea mariana* (Mill.) BSP and *Abies balsamea* (L.) Mill.) of North American trees (Walters and Reich, 2000), where high plasticity indices were observed in larger seeds in few analyzed variables. This can be attributed to the factors evaluated in the experiment (N content and seeding depth). The plasticity results showed that smaller seeds had higher growth and development capacity than medium and larger seeds, so they have a greater ecological advantage as they can be widely distributed at different sites (Moles and Westoby, 2004), *i.e.*, a higher value of root biomass indicates that seeds are more drought resistant (Olmo *et al.*, 2014) and may have a better survival rate at sites with low moisture availability. However, these results differ from those reported by Miniño *et al.* (2014), who elucidated that a large seed would give a larger seedling with a higher probability of survival as compared to a smaller one. For the stem dry biomass, *Q. variabilis* recorded lower values (18.56 to 29.78) compared to pine species (35.04 to 43.03) recorded by Flores *et al.* (2018) using the same phenotypic plasticity index.

According to the allometric analysis, the regression model showed differences in both intercept and slope, *i.e.*, the null hypothesis (H_0) was rejected at a 0.05 significance level. Comparing the regression coefficients of the generated models (root dry biomass=1.8374+4.0943*stem dry biomass and root dry biomass=3.4956+1.7038*stem dry biomass; unfertilized and fertilized seedlings, respectively), the expected root dry biomass was approximately 2.4-fold higher in unfertilized seedlings with respect to the fertilized seedlings. In other words, an increase of 1.7038 and 4.0943 units in root dry biomass is expected for each unit increase in stem dry biomass in fertilized and unfertilized seedlings, respectively.

Application of fertilizer on *Q. variabilis* seedlings promoted changes in biomass allocation, probably due to the capacity of the seedlings to alter their nutrient reserves by imposing stimulus (fertilization) during early stages of development (Bloom *et al.*, 1985). This condition showed that the fertilization routine applied affected biomass production, which is not always the case, *e.g.*, in a *Populus maximowiczii* A. Henry × *P. balsamifera* L. hybrid, no significant effect was noted on the biomass allocation. According to the specialization theory (Lortie and Aarssen, 1996), genotypes adapted to favorable conditions will perform better in benign environment, which was observed in *Q. variabilis* found at sites with good nutrient supply.

Non-parametric multivariate analysis of the data based on Canonical Discriminant Analysis revealed that the first two canonical components were significant (p-value<0.0002) and explained 98.80% of the total variability. The first canonical component explained 95.31%, where the main phenotypic traits were root dry biomass and stem dry biomass; while the second canonical component explained only 3.49%, and the most important phenotypic traits were root dry biomass and stem dry biomass (Table 4) by presenting the largest weights in both components. However, only the first canonical component was considered because it explained more than 95% of the total variability, and the most important phenotypic traits were root dry biomass and stem dry biomass in both components.

The analysis of the groups formed according to the weights of the first canonical component (Table 4) showed that groups 3 and 6 were characterized by high root dry biomass as the canonical component value was negative, and with less intensity, the groups 1 and 2 also were characterized by the root dry biomass. In contrast, groups 4 and 5 differed by presenting larger heights, diameters, and stem dry biomass and a small root dry biomass such that the value of the canonical component was positive (Table 4). As in the previous case, groups 1 and 2 presented a positive canonical component value (Table 3), this indicated that groups 1 and 2 were more unstable than the rest by presenting positive and negative values of the canonical component.

According to the results of the canonical discriminant analysis, the phenotypic traits of root dry biomass and stem dry biomass were the most important since they had the highest proportion of variability compared to the remaining attributes, so these variables were most important for grouping individuals. In this regard, it is to be expected —in young *Q. variabilis* seedlings— that during routine application of fertilization, the variables associated with biomass showed a greater response than those related to growth, *i.e.*, height and diameter. These results corroborate with those reported for root dry biomass and stem dry biomass in *Q. variabilis* (Li *et al.*, 2014; Wang *et al.*, 2016), and in *Agathis australis* (D. Don) Lindl., *Dacrycarpus dacrydiodes* (A. Rich.) de Laub., *Knightia excelsa* R. Br. and *Laurelia novae-zelandiae* A. Cunn (Kramer-Walter and Laughlin, 2017).

The root biomass is an important aspect of *Q. variabilis* since the characteristics associated with the root abundance and quality indicate that seedlings have greater survival rate (Grossnickle, 2012) and growth potential (Grossnickle and MacDonald, 2018) in fields.

Also, the canonical dispersion plot showed a marked difference between fertilized and unfertilized seedlings; also, unfertilized and seedlings produced from medium and smaller seeds presented a more heterogeneous behavior than the remaining ones. This implies that for *Q. variabilis*, the variation is influenced by seed size, which could be related to the number of nutritional reserves accumulated in the seeds, as is the case of *Q. glauca* Thunb. (Negi and Rawal, 2018).

Table 4. Total explained variance percentage and values of canonical coefficients.

Variable†	Canonical components	
	CC1 (95.31%)	CC2 (3.49%)
H	0.31140	0.41100
D	0.17537	0.12674
RDB	−0.43547	1.35510
SDB	2.15264	−1.12208

†H: Height (cm), D: Root collar diameter (mm), RDB: Root dry biomass ($g \cdot seedling^{-1}$), SDB: Stem dry biomass ($g \cdot seedling^{-1}$).

According to the Mahalanobis distances, most of the distances were significant at p-value<0.05 because there were differences between groups in the lower triangular matrix (Table 5) and p-values<0.05 (upper triangular matrix). It was possible to identify the different groups defined between fertilized and unfertilized seedlings, which were produced in different seed sizes (small, medium and large), except for the distance between the groups defined by unfertilized seedlings produced with larger seeds (Group 1) and fertilized seedlings produced with medium seed (Group 5) (p-value=0.2442).

Table 5. Mahalanobis distance matrix between groups defined by fertilized and unfertilized seedlings produced with three seed sizes of *Quercus variabilis*.

Group	Group†					
	1	**2**	**3**	**4**	**5**	**6**
1	0	0.0087	0.0001	0.0001	0.2131	0.0001
2	1.157*	0	0.0001	0.0001	0.0001	0.0001
3	14.9725*	9.430*	0	0.0001	0.0001	0.0001
4	8.156*	13.851*	41.671*	0	0.0001	0.0001
5	0.4811ns	2.406*	19.541*	4.875*	0	0.0001
6	4.123*	1.983*	3.478*	23.006*	7.130*	0

†1: Non-fertilized plant with large seed, 2: non-fertilized plant with medium seed, 3: non-fertilized plant with small seed, 4: fertilized plant with large seed, 5: fertilized plant with medium seed and 6: fertilized plant with small seed. *: Significant difference (p-value≤0.05), ns: non-significant difference.

CONCLUSIONS

The above results showed that phenotypic plasticity in *Q. variabilis* was observed between fertilized and unfertilized seedlings when they were produced from small, medium, and large seeds. It is suggested to use medium and large seeds during nursery plant production and to establish fertilized plants in the field because they may have a greater advantage in survival and growth than unfertilized seedlings.

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REFERENCES

Agathokleous, E., Kitao, M., Tamai, Y., Harayama, H. & Koike, T. (2022). Single and combined effects of fertilization, ectomycorrhizal inoculation, and drought on container-grown Japanese larch seedlings. *Journal of Forestry Research*, 34, 1077-1094. Doi: 10.1007/s11676-022-01565-3

Bloom, A. J., Chapinand, F. S. & Mooney, H. A. (1985). Resource limitation in plants-an economic analogy. *Ann. Rev. Ecol. Syst.*, 16, 363-392. Doi: 10.1146/annurev.es.16.110185.002051

Clark, S. L. & Schlarbaum, S. E. (2018). Effects of acorn size and mass on seedling quality of northern red oak (*Quercus rubra*). *New Forests, 49*(4), 571-583. Doi: 10.1007/s11056-018-9641-9

Costa, J. T. (2021). There is hardly any question in biology of more importance: Charles Darwin and the nature of variation. In: D. W. Pfenning (Ed.), Phenotypic plasticity & evolution: causes, consequences, controversies (pp. 49-72). CRC Press, Florida, USA.

Dziedek, C., Fichtner, A., Calvo, L., Marcos, E., Jansen, K., Kunz, M., Walmsley, D., von Oheimb, G. & Hardtle, W. (2017). Phenotypic plasticity explains response patterns of European beech (*Fagus sylvatica* L.) saplings to nitrogen fertilization and drought events. *Forests*, 8, 91. Doi: 10.3390/f8030091

Feng, Y., Li, X., Zhou, B., Hu, R., Zhang, H., Yang, J. & Fang, Y. (2021). Inter- and intra-specific phenotypic variation of ecological stoichiometric traits in a mixed-oak secondary forest in China. *J. For. Res*., 32, 2333-2347. Doi: 10.1007/s11676-021-01304-0

Flores, A., Climent, J., Pando, V., López-Upton, J. & Alía, R. (2018). Intraspecific variation in pines from the Trans-Mexican Volcanic Belt grown under two watering regimes: Implications for management of genetic resources. *Forests*, 9, 71. Doi: 10.3390/f9020071

Gao, W.-Q., Liu, J.-F., Xue, Z.-M., Zhang, Y.-T., Gao, Z.-H., Ni, Y.-Y., Wang X.-F. & Jiang, Z.-P. (2018). Geographical patterns and drivers of growth dynamics of *Quercus variabilis*. *Forest Ecology and Management*, 429, 256-266. Doi: 10.1016/j.foreco.2018.07.024

Grossnickle, S. C. (2012). Why seedlings survive: influence of plant attributes. *New Forests, 43*(5-6), 711-738. Doi: 10.1007/s11056-012-9336-6

Grossnickle, S. C. & MacDonald, J. E. (2018). Why seedlings grow: influence of plant attributes. *New Forests,* 49, 1-34. Doi: 10.1007/s11056-017-9606-4

Hernández-Pérez, C., Vargas-Hernández, J. J., Ramírez-Herrera, C. & Muñoz-Orozco, A. (2001). Variación geográfica en la respuesta a la sequía en plántulas de *Pinus greggii* Engelm. *Rev. Ciencia Forestal en México, 26*(89), 61-79. http://cienciasforestales.inifap.gob.mx/index.php/forestales/article/view/919

Kramer-Walter, K. R. & Laughlin, D. C. (2017). Root nutrient concentration and biomass allocation are more plastic than morphological traits in response to nutrient limitation. *Plant Soil, 416*(1-2), 539-550. Doi: 10.1007/s11104-017-3234-9

Li, Y., Li, S., Lu, X., Wang, Q., Han, H., Zhang, X., Ma, Y. & Gan, X. (2021). Leaf phenotypic variation of endangered plant Tetracentronsinense Oliv. and influence of geographical and climatic factors. *J. For. Res., 32*(2), 623-636. Doi: 10.1007/s11676-020-01124-8

Li, G., Zhu, Y., Liu, Y., Wang, J., Liu, J. & Dumroese, R. K. (2014). Combined effects of pre-hardening and fall fertilization on nitrogen translocation and storage in *Quercus variabilis* seedlings. *European Journal of Forest Research, 133*(6), 983-992. Doi: 10.1007/s10342-014-0816-4

Lortie, C. J. & Aarssen, L. W. (1996). The specialization hypothesis for phenotypic plasticity in plants. Int. *J. Plant Sci., 157*(4), 484-487. Doi: 10.1086/297365

Mardia, K. V. (1970). Measures of multivariate skewness and kurtosis with applications. *Biometrika, 57*(3), 519-530. Doi: 10.1093/biomet/57.3.519

Mechergui, T., Pardos, M. & Jacobs. D. F. (2021) Effect of acorn size on survival and growth of *Quercus suber* L. seedlings under water stress. *European Journal of Forest Research, 140*(1), 175-186. Doi: 10.1007/s10342-020-01323-2

Miniño, M. V. A., Rodríguez, F. L. E., Paíno, P. O., León, Y. and Paulino, L. (2014). Caracterización de la morfología de la semilla de *Pinus occidentalis* Swartz. *Ciencia y Sociedad, 39*(4), 777-801. Doi: 10.22206/cys.2014.v39i4.pp777-801

Moles, A. T. & Westoby, M. (2004). What do seedlings die from and what are the implications for evolution of seed size? *Oikos, 106*(1), 193-199. Doi: 10.1111/j.0030-1299.2004.13101.x

Montgomery, D. C., Peck, E. A. & Vining, G. G. (2012). Introduction to linear regression analysis. John Wiley & Sons. Hobokem, New Jersey, USA.

Negi, M. & Rawal, R. (2018). Effects of pre-sowing treatments on seed germination of oaks in Kumaun, West Himalaya. *Not. Sci. Biol*., 10, 282-286. Doi: 10.25835/nsb10210240

Oksanen, E., Lihavainen, J., Keinänen, M., Keski-Saari, S., Kontunen-Soppela, S., Sellinand, A. & Sõber, A. (2018). Northern forest trees under increasing atmospheric humidity. In: F. Cánovas, U. Lüttge, R. Matyssekand & H. Pretzsch (Eds.), Progress in Botany (pp. 317-336). Springer Berlin Heidelberg, Heidelberg, Germany. Doi: 10.1007/124_2017_15

Olmo, M., Lopez-Iglesias, B. & Villar, R. (2014). Drought changes the structure and elemental composition of very fine roots in seedlings of ten woody tree species. Implications for a drier climate. *Plant Soil, 384*(1-2), 113-129. Doi: 10.1007/s11104-014-2178-6

Pająk, K., Małek, S., Kormanek, M. & Jasik, M. (2022). The effect of peat substrate compaction on the macronutrient content of Scots pine *Pinus sylvestris* L. container seedlings. *Sylwan, 166*(3), 211-233. Doi: 10.26202/sylwan.2022023

Pazzaglia, J., Reusch, T. B. H., Terlizzi, A. & Marín-Guirao, L. (2021). Phenotypic plasticity under rapid global changes: The intrinsic force for future seagrasses survival. *Evol. Appl., 14*(5), 1181-1201. Doi: 10.1111/eva.13212

Pigliucci, M. & Schlichting, C. D. (1996). Reaction norms of Arabidopsis. IV. Relationships between plasticity and fitness. *Heredity*, 76, 427-436. Doi: 10.1038/hdy.1996.65

R Core Team. (2015). R: A language and environment for statistical computing [Computer software]. R Foundation for Statistical Computing, Vienna, Austria. Disponible en: http://www.RProject.org/

Rivero, R. M., Kojima, M., Gepstein, A., Sakakibara, H., Mittler, R., Gepstein, S. & Blumwald, E. (2007). Delayed leaf senescence induces extreme drought tolerance in a flowering plant. *PNAS, 104*(49): 19631-19636. Doi: 10.1073/pnas.0709453104

Shi, W., Villar-Salvador, P., Li, G. & Jiang, X. (2019). Acorn size is more important than nursery fertilization for outplanting performance of *Quercus variabilis* container seedlings. *Annals of Forest Science, 76*(1), 22. Doi: 10.1007/s13595-018-0785-8

Sun, S., Zhang, Y., Huang, D., Wang, H., Cao, Q., Fan, P., Yang, N., Zheng, P. & Wang, R. (2020). The effect of climate change on the richness distribution pattern of oaks (*Quercus* L.) in China. *Science of the Total Environment*, 744, 140786. Doi: 10.1016/j.scitotenv.2020.140786

Vinha, A. F., Barreira, J. C. M., Costa, A. S. G. & Oliveira, M. B. P. P. (2016). A new age for *Quercus* spp. fruits: Review on nutritional and phytochemical composition and related biological activities of acorns. *Comprehensive Reviews in Food Science and Food Safety, 15*(6), 947-981. Doi: 10.1111/1541-4337.12220

Vizcaíno-Palomar, N., Garzón, M. B., Alia, R., Giovannelli, G., Huber, G., Mutke, S., Pastuszka, P., Raffin, A., Sbay, H., Šeho, M., Vauthier, D. & Fady, B. (2019). Geographic variation of tree height of three pine species (*Pinus nigra* Arn., *P. pinaster* Aiton, and *P. pinea* L.) gathered from common gardens in Europe and North-Africa. *Annals of Forest Science, 76*(3), 6. Doi: 10.1007/s13595-019-0867-2

Walters, M. B. & Reich, P. B. (2000). Seed size, nitrogen supply, and growth rate affect tree seedling survival in deep shade. *Ecology, 81*(7), 1887-1901. Doi: 10.1890/0012-9658(2000)081[1887:SSNSAG]2.0.CO;2

Wang, J., Yu, H., Li, G. & Zhang, F. (2016). Growth and nutrient dynamics of transplanted *Quercus variabilis* seedlings as influenced by prehardening and fall fertilization. *Silva Fennica, 50* (2), 1-18. Doi: 10.14214/sf.1475

Wu, M., Zhang, W.-H., Zhou, J.-Y., Maand, C. & Ma, L.-W. (2013). Seedling regeneration and affecting factors of *Quercus variabilis* in different distribution regions. *Chinese Journal of Applied Ecology, 24*(8), 2106-2114. https://www.researchgate.net/publication/259529658_Seedling_regeneration_and_affecting_factors_of_Quercus_variabilis_in_different_distribution_regions/citations

Xu, N., Guo, W., Liu, J., Du, N. & Wang, R. (2015). Increased nitrogen deposition alleviated the adverse effects of drought stress on *Quercus variabilis* and *Quercus mongolica* seedlings. *Acta Physiol. Plant., 37*(6), 1-11. Doi: 10.1007/s11738-015-1853-4

Yadegari, L. Z. & Seyedi, N. (2019). Effect of altitude on seed germination and biomass of *Quercus brantii*. *Journal of Forest Research and Development, 5*(3), 405-417. Doi: 10.30466/jfrd.2019.120784

Zhang, W., Lu, Y., Zhou, J., Zhang, X. & Shi, X. (2008). Population structure and dynamics of *Quercus variabilis* in different habitats on northern slope of Bashan mountain. *Sci. Silvae Sinicae, 44*(7), 11-16. Doi: 10.11707/j.1001-7488.20080703

Colegio de Postgraduados

Effects of early and late sowing on the profitability of cucumber (*Cucumis sativus* L.) production

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ABSTRACT
Objective: To analyze how early and late sowing can improve the income of cucumber producers in the state of Michoacán, Mexico.
Design/Methodology/Approach: Monthly income, production cost, profit, and benefit-cost ratio were calculated in three sowing scenarios: middle, early, and late.
Results: In an early sowing scenario, the income and profit of the producers increase by 10.2 and 9.2 million pesos, respectively, compared to the middle sowing scenario. Likewise, in a late sowing scenario, producer income and profits increase by 29.2 and 20.0 million pesos, regarding the middle sowing scenario.
Study Limitations/Implications: The negative effects on vegetable prices of temporary excess supply prove the need to plan the surface that will be used for early and late sowing, as well as to create a regulatory body that plans the cucumber sowing area.
Findings/Conclusions: Sowing cucumber and other vegetables in early or late dates is recommended, since better production planning will increase the producers' income and profit.

Keywords: cucumber, early sowing, late sowing, planning.

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INTRODUCTION

Cucumber (*Cucumis sativus* L.) is one of the main vegetables exported by Mexico to the global market. For example, in 2020 Mexico was the largest exporter of cucumbers of the Americas, with 809,000 tons (FAO, 2020). Michoacán is the fourth largest cucumber producer in the country, behind the states of Sinaloa, Sonora, and Morelos (SIAP, 2021). In 2021, it accounted for 17.1% of the domestic market (Martínez-Mendoza, 2021). The cucumbers produced in Michoacán are destined for the domestic market (SADER, 2018). In 2020, Michoacán produced 64,900 tons of cucumber. This was an atypical year, in terms of harvest and commercialization, since this was the lowest volume for the 2016-2020 period, when the production reached >95,000 tons each year (SIAP, 2022b).

Like most horticultural products, cucumber faces a temporary price volatility problem. The fall in prices is a consequence of several factors, including the seasonality of production (Martínez-Jiménez and García-Salazar, 2020). Since cucumber production depends on biological and climatic conditions, it is concentrated in certain months. On the one hand, the largest cucumber harvest was obtained in January 2020, with 12,000 tons (SIAP, 2022a) and the producer price was the lowest of the year, with 3,500 pesos per ton (SIAP, 2022b). In conclusion, the decrease in income negatively impacts the producers.

On the other hand, volatility implies that cucumbers are more expensive in some months, as a consequence of the scarcity of the product. For example, the producer price reached $6,500 pesos per ton in April, a month in which barely 3,000 tons were produced (Table 1). In this sense, high cucumber prices have a negative effect on the consumer.

Another factor that explains the fall in cucumber prices is that most vegetables are perishable, which makes their storage impossible. Unlike grains, vegetables cannot be stored and, if they are consumed fresh, it must be shortly after they have been harvested. A third factor is the atomization of cucumber production. In the case of Michoacán, 970 agricultural producers are focused on this activity, which determines a high geographic dispersion and low price-negotiation power.

The inverse relationship between production and price accentuates the problem. The production cost of cucumber can reach $105,000 mexican pesos per ha (Ramírez Abarca *et al.*, 2021), while an average yield of 13.9 tons per ha was obtained in Michoacán (SIAP, 2021). The previous data indicate that the cost can reach $7,600 pesos per ha —higher than the price in some harvest months.

The seasonal fall in prices caused by excess supply is a phenomenon that has been recorded in many countries. Since 1937, the USA has addressed the problem through a marketing order policy, which focuses on the organization of the vegetable and fruit markets, through the supervision and regulation of trade agreements (AMS, 2022).

In Canada, the government pays a compensation to the producers, if the market price of their product does not reach the target; in exchange, farmers agree to refrain from sowing when the market is saturated (Colomé, 2010).

Currently, Mexico lacks a similar policy. In the 1990s, the excessive supply of horticultural products and the price volatility in Mexico were addressed by the Confederación Nacional de Productores de Hortalizas (CNPH), which analyzed the most important vegetables, as well as the future needs of the global market (Ramírez-Barraza *et al.*, 2015).

If the volatility of cucumber prices in Michoacán is a consequence of the seasonality of production, then the problem can be faced by "moving" production over time. Increasing the synchronization of early and late sowing with consumption will prevent temporary excess supply and falling prices.

The aim of early and late sowing would be to increase the producers' income. However, the implementation of these practices poses risks, such as frost or the presence of pests and diseases (Chew Madinaveitia, 2009).

This research sought to analyze the effects that early and late sowing would have on the producers' income and profit. The hypothesis establishes that early and late sowing will improve the income of Michoacán's cucumber producers.

MATERIALS AND METHODS

To achieve the objective, the producers' income, cost, and profit were calculated in different scenarios, considering early and late sowing. Using t as the month in which the production is obtained, the following indicators were determined:

$$ING_t = QPP_t * PP_t \tag{1}$$

$$CP_t = \sum_{i=1}^{I}\left[X_{it} * PI_{it}\right] \tag{2}$$

$$G_t = ING_t - CP_t \tag{3}$$

$$GU_t = \frac{G_t}{QPP_t} \tag{4}$$

$$RBC_t = \frac{G_t}{CP_t} \tag{5}$$

Where ING_t is the income for month t; QPP_t is cucumber production for month t; PP_t is the average rural price for month t; CP_t is the production cost for month t; X_{it} is the quantity of input i used for month t; PI_{it} is the price of input i for month t; G_t is the profit for month t; GU_t is the unit profit for month t; and RBC_t is the Benefit-Cost Ratio for month t.

Income (Equation 1) is determined multiplying production by the average rural price. Production costs (Equation 2) are the results of adding the partial costs (quantity of inputs multiplied by the price of each input). The monthly profit (Equation 3) is obtained discounting production costs from income. The unit profit (Equation 4) is determined through the division of the total monthly profit by the monthly production. The Benefit-Cost Ratio per month was established using Equation 5.

To achieve the objective, income and profit were calculated in three scenarios: a) observed sowing in the year of analysis (middle sowing), b) early sowing, and c) late sowings. Cucumber production in Michoacán in 2020 was obtained from SIAP (2022b). The average rural cucumber prices were obtained from the Sistema de Información Agroalimentaria de Consulta (SIAP, 2022b). The cucumber production cost per hectare was calculated using the monthly yield reported in the sowing and harvesting estimates (SIAP, 2021) and the production cost per hectare was obtained from Ramírez Abarca (2021).

The agricultural calendar reported by the Secretaría de Agricultura y Desarrollo Rural (SADER, 2022) was used for this study. According to this calendar, the AW sowing cycle starts on October of one year and ends on March of the following year, while the harvests begin in December of one year and conclude in September of the following year. Meanwhile, the SS sowing cycle occurs from April to September of one year and the harvests from June to March of the following year.

RESULTS AND DISCUSSION

Middle sowings

In 2020, the annual cucumber production in Michoacán was 65,000 tons. The monthly production ranged from 2,300 to 12,000 tons and extreme values were reported in December and January. In conclusion, a market seasonality was observed. The results are shown in Table 1.

The average annual price was $4,457 pesos per ton, although production seasonality impacted price behavior, which fluctuated between $3,476 and $6,460 pesos per ton (Table 1).

The producers' income amounted to $280.9 million pesos and costs reached $287.4 million pesos, resulting in a loss of $6.5 million pesos. Since income depends on the behavior of production and price, a strong variation is observed between the minimum ($9.0 million pesos in December) and the maximum ($41.8 million pesos in January) values. The producers' economic benefit also ranged from $16.4 to $5.7 million pesos between March and September (Table 1). The average monthly Benefit-Cost Ratio (RBC) was 0.98, which indicates that $2 pesos were lost for every $100 pesos invested.

Early sowing

Sowing, particularly during the early stages, optimizes the number of plants harvested, maximizing production, as long as the amount of water, physical barriers, and soil

Table 1. Income and profits from cucumber production in Michoacán, Mexico in 2020. Thousands of tons, pesos per ton, and millions of pesos.

Month	Production thousands of tons	Price pesos per ton	Income	Cost	Profits		Benefit-cost ratio
					total	unit	
			millions of pesos		pesos per ton		
January	12.0	3,476	41.8	43.6	−1.72	−143	0.96
February	9.5	4,118	39.3	34.3	5.00	523	1.15
March	7.4	5,351	39.8	56.2	−16.38	−2,201	0.71
April	3.0	6,460	19.5	14.2	5.36	1,774	1.38
May	3.2	5,597	18.2	14.4	3.79	1,168	1.26
June	4.0	4,643	18.3	17.3	1.07	270	1.06
July	4.6	3,689	17.0	19.9	−2.98	−648	0.85
August	4.1	3,775	15.6	18.0	−2.33	−562	0.87
September	5.3	4,028	21.4	15.7	5.69	1,071	1.36
October	6.1	4,656	28.3	27.8	0.56	93	1.02
November	3.3	3,760	12.5	16.1	−3.58	−1,077	0.78
December	2.3	3,936	9.0	10.0	−1.00	−438	0.90
Total	65.0	4,457	280.9	287.4	−6.52	−100	0.98

Source: Table developed by the authors, based on the results of Scenario 1.

nutrients are controlled. Table 2 shows the effect on profit of implementing early sowing. The scenario was based on the same annual cucumber production in Michoacán (65,000 tons), distributed in a different proportion each month. The same average rural price was considered. The results of the scenario are: a) the producers' income increased from $280.9 to $291.0 million Mexican pesos; b) total profit increased from -$6.5 to $2.6 million pesos; c) the profit per ton increased from -$100 to $41 pesos per ton and; d) the benefit-cost ratio increased from 0.98 to 1.01. These changes in production over time can be observed by comparing Table 1 and 2.

Late sowing

Late sowing during the AW cycle is carried out in the months of February and March, which allows producers to adjust harvest times to coincide with the months with highest prices (April and May). Therefore, delaying part of the sowing from January to May, allowed producers to benefit from the higher prices recorded in 2020.

The results obtained with the late sowing scenario are the following: a) income increased from $280.9 to $310.1 million pesos, which represents a 10.4% growth, compared with the base scenario; b) the total profit increased by $20.0 million pesos, from −$6.5 to $13.5 million pesos; and d) the unit profit increased from −$100 to $207 pesos per ton, much higher than the increase observed in the early sowing scenario. The improvement of the benefit-cost ratio amounted to 1.05.

The results obtained in the two scenarios are similar to those obtained by other authors. Espinoza-Arellano (2019) determined that profitability was higher in the early and late sowing of melon of 2016 than in the average sowing. Grijalva Contreras (2011) concludes

Table 2. Income and profit from cucumber production in Michoacán, Mexico in an early sowing scenario. Thousands of tons, pesos per ton, and millions of pesos.

Month	Production thousands (ton)	Price pesos per ton	Income	Cost	Profits		Benefit-cost ratio
					total	unit	
			millions of pesos		pesos per ton		
January	12.0	3,476	41.8	43.6	−1.72	−143	0.96
February	9.5	4,118	39.3	34.3	5.00	523	1.15
March	7.4	5,351	39.8	56.2	−16.38	−2,201	0.71
April	5.4	6,460	35.0	25.4	9.62	1,774	1.38
May	5.0	5,597	28.0	22.2	5.85	1,168	1.26
June	4.2	4,643	19.6	18.5	1.14	270	1.06
July	1.5	3,689	5.7	6.6	−0.99	−648	0.85
August	2.8	3,775	10.4	12.0	−1.55	−562	0.87
September	5.3	4,028	21.4	15.7	5.69	1,071	1.36
October	6.1	4,656	28.3	27.8	0.56	93	1.02
November	3.3	3,760	12.5	16.1	−3.58	−1,077	0.78
December	2.3	3,936	9.0	10.0	−1.00	−438	0.90
Total	65.0	4,457	291.0	288.4	2.64	41	1.01

Source: Table developed by the authors, based on the results of Scenario 2.

Table 3. Income and profit from cucumber production in Michoacán, Mexico in a late sowing scenario. Thousands of tons, pesos per ton, and millions of pesos.

Month	Production thousands (ton)	Price pesos per ton	Income	Cost	Profit		Benefit-cost ratio
					total	unit	
			millions of pesos		pesos per ton		
January	4.0	3,476	13.9	14.5	−0.57	−143	0.96
February	4.8	4,118	19.7	17.2	2.50	523	1.15
March	6.5	5,351	34.7	49.0	−14.29	−2,201	0.71
April	9.9	6,460	63.9	46.3	17.54	1,774	1.38
May	9.0	5,597	50.6	40.1	10.56	1,168	1.26
June	5.0	4,643	23.4	22.0	1.36	270	1.06
July	4.6	3,689	17.0	19.9	−2.98	−648	0.85
August	4.1	3,775	15.6	18.0	−2.33	−562	0.87
September	5.3	4,028	21.4	15.7	5.69	1,071	1.36
October	6.1	4,656	28.3	27.8	0.56	93	1.02
November	3.3	3,760	12.5	16.1	−3.58	−1,077	0.78
December	2.3	3,936	9.0	10.0	−1.00	−438	0.90
Total	65.0	4,457	310.1	296.6	13.47	207	1.05

Source: Table developed by the authors, based on the results of Scenario 3.

that, by delaying its sowing, cucumber can be produced from November to May, dates that match the months of 2020 —when the highest producer prices were obtained (March, April, and May).

Although early and late sowings allow producers to face the volatility of cucumber prices, they also imply certain risks. Early AW sowings and harvests —which occur from November to the end of January— offer the producers the possibility of accessing better prices, at the risk of having their plants die from frost (Espinoza-Arellano, 2019). To avoid these and other agricultural risks, Bielinski *et al.* (2010) point out that crop protection during winter is a modern measure. Artificially altering the microclimate changes solar radiation, temperature, wind, humidity, and soil conditions, providing protection against frost and strong winds, extending crop cycles, and promoting precocity (advancement of the harvest).

The drawback of late SS sowing and late AW harvests —which take place in summer and spring, respectively— are high temperatures, that favor the presence of insects, fungi, and viruses (Espinoza-Arellano, 2019). According to Arias and Fuentes (2012), the phytosanitary aspect has a high incidence in cucumber production, especially in late sowing, when fungi, bacteria, viruses, plasmodia, weeds, and insects attack the plant to use it as a means of reproduction or food, potentially damaging and affecting the development of the crop.

Monitoring the crop and establishing integrated pest management (*e.g.*, rotating cucumber with sweet pepper) is an important measure to address this problem. Some diseases caused by microorganisms impact the cucumber's quality of production, damaging the leaves, vascular system, roots, and fruit. Pest and disease management requires a logic of integrity. Phytosanitary control must be considered as a unit of simultaneous protection, which includes the reduction of the environmental impact and the sustainability of the agroecosystem. The implementation of a given measure should contribute to solve another core problem.

Mexican producers must, as in other countries, join a regulatory body to address price variability. Among other functions, the said body would carry out economic studies to determine the optimal production levels that meet domestic and international consumers. In a joint effort with the Mexican Ministry of Agriculture, quotas should be established to avoid temporary excess supply and market saturation.

This organization must include experts and specialized academicians to carry out economic research that promotes the marketing order. The organization will be an agricultural regulatory body that organizes agricultural markets to control the supply of products in periods of abundance. It should also promote technologies such as staged sowing.

CONCLUSIONS

The analysis of the income and costs of cucumber production in Michoacán indicates that seasonality prevents the profitability of the said activity during certain months. Price volatility can be addressed through early and late sowing. Such practices —based on the modification of sowing and harvest times to take advantage of the months in which average rural prices exceed the annual average— allow the farmers to move from a loss situation to a profit situation. In early and late sowing scenarios, the benefit-cost ratio would increase from 0.98 to 1.01 and 1.05. Therefore, producers are advised to choose a late sowing scenario.

The excesses in the temporary supply of cucumber cause the fall in prices and can only be avoided through the organization of the producers. They must jointly plan the level of production and surface that allows them to supply the domestic market and avoid its saturation.

REFERENCES

AMS (Agricultural Marketing Service) (2022) Commodities covered by marketing orders. Washington, D.C., USA. Recuperado de https://www.ams.usda.gov/rules-regulations/tools-fruits-vegetables-specialty-crops

Arias L. y L. Fuentes (2012) Manual de producción de pepino bajo invernadero. Bogotá, Colombia. Recuperado de http://hdl.handle.net/20.500.12324/34357.

Bielinski S., H. Obregón-Olivas y T. Salamé-Donoso (2010). Producción de hortalizas en ambientes protegidos: estructuras para la agricultura protegida. Recuperado de https://horticulture.ucdavis.edu/sites/g/files/dgvnsk1816/files/extension_material_files/Santos_academic_paper_estructuras_para_la_agricultura_protegida.pdf

Colomé, R. A. (2010) Política agrícola canadiense, algunas lecciones para argentina. *Actualidad Económica XX* (71):27-45. Recuperado de https://revistas.unc.edu.ar/index.php/acteconomica/article/view/3894/3729

Chew Madinaveitia, Y. I., A. Gaytán Mascorro, C. Serrano Gómez, y U. Nava Camberos (2009) Manejo del virus del amarillamiento y achaparramiento de las cucurbitáceas en el cultivo de melón. *Revista Chapingo Serie Zonas Áridas VIII*(2):105-108. Recuperado de https://www.redalyc.org/articulo.oa?id=455557520001

Espinoza-Arellano, J. J. I. Orona-Castillo, L. A. Guerrero-Ramos, V. M. Molina-Morejón y E. C. Ramírez-Quiroga (2019). Análisis del financiamiento, comercialización y rentabilidad del cultivo del melón con enfoque de “siembras por etapas” en la Comarca Lagunera de Coahuila, México. *Ciencia UAT 13*(2):71-82. Recuperado de https://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S2007-78582019000100071#B8

FAO (Organización de las Naciones Unidas para la Alimentación y la Agricultura) (2020). Matriz detallada del comercio, FAOSTAT, Roma, Italia. Recuperado de https://fenix.fao.org/faostat/internal/es/#data/TM

Grijalva Contreras R. L., R. Macías Duarte, S. A. Grijalva Durón y F. Robles Contreras (2011). Evaluación del efecto de la fecha de siembra en la productividad y calidad de híbridos de pepino europeo bajo condiciones de invernadero en el Noroeste de Sonora. *BIOtecnia XIII*(1):29-36. Recuperado de https://biblat.unam.mx/hevila/Biotecnia/2011/vol13/no1/5.pdf

Martínez-Jiménez, A. y J. A. García-Salazar (2020) Volatilidad de precios en el sector frutícola de México: El caso de la naranja. *Acta Universitaria 30*(1):1-14. Recuperado de https://doi.org/10.15174/au.2020.2513

Martínez-Mendoza, A. J. (2021) Estudio de mercado, función de costos, canales y márgenes de comercialización del pepino persa (*Cucumis sativus* L.) de la Empresa Gromich S.P.R de R.L. de C.V. a Estados Unidos. Texcoco, Estado de México https://repositorio.chapingo.edu.mx/items/66e01a41-0d83-4b7b-8deb-d39c26bdba21

Ramírez Abarca, O., J. Hernández Martínez y F. de J. González Razo (2021) Análisis económico del pepino persa en condiciones de invernadero en Guerrero y estado de México, 2020. *Revista Mexicana de Agronegocios* 48:678-689. Recuperado de https://www.redalyc.org/journal/141/14167610009/html/

Ramírez Barraza, B. A., J. A. García-Salazar y J. S. Mora-Flores (2015) Producción de melón y sandía en la Comarca Lagunera: un estudio de planeación para reducir la volatilidad de precios. *Ciencia ergo-sum 22*(1):45-53. Recuperado de https://cienciaergosum.uaemex.mx/article/view/7845

SADER en Michoacán (Secretaría de Agricultura y Desarrollo Rural en Michoacán) (2018) El Cultivo de pepino en Michoacán rebasa los 400 mdp en valor de producción. Morelia, Michoacán. Recuperado de https://www.gob.mx/agricultura/michoacan/articulos/el-cultivo-de-pepino-en-michoacan-rebasa-los-400-mdp-en-valor-de-produccion?idiom=es

SADER (Secretaría de Agricultura y Desarrollo Rural) (2022) Calendario agrícola mexicano: una guía para las cosechas. Ciudad de México, México. Recuperado de https://www.gob.mx/agricultura/es/articulos/calendario-agricola-mexicano-una-guia-para-las-cosechas?idiom=es

SIAP (Servicio de Información Agroalimentaria y Pesquera) (2021) Avance de siembras y cosechas. Ciudad de México, México. Recuperado de https://nube.siap.gob.mx/avance_agricola/

SIAP (Servicio de Información Agroalimentaria y Pesquera) (2022a) Estacionalidad por año agrícola. Ciudad de México, México. Recuperado de http://infosiap.siap.gob.mx/estacionalidad_gb/est_agricola-AA/index.php

SIAP (Servicio de Información Agroalimentaria y Pesquera) (2022b) Sistema de Información Agroalimentaria de Consulta. Ciudad de México, México.https://www.gob.mx/siap/documentos/siacon-ng-161430

Effect of harvest date on botanical, morphological, and nutritional composition of mixed crops of small-grain cereals for silage

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ABSTRACT

Objective: To evaluate the effect of harvest date on the botanical, morphological and nutritional composition of silage from small grain cereal mixtures.

Design/methodology/approach: Laboratory silages of three crops of small grain cereal mixtures (BR, barley +rye; BT, barley+triticale and RT, rye+triticale) were made on two harvest dates (HD1, 60 days and HD2, 80 days post-sowing). Statistical analysis was performed under a 3×2 factorial model, and the variables were botanical, morphological and nutritional composition.

Results: The proportion of cereal decreased from HD1 to HD2 ($p<0.05$). Spikes and stems in barley and triticale increased in HD2. Rye had a high proportion of stems on both dates. Crude protein (CP) decreased and neutral detergent and acid detergent fiber increased in HD2 ($p<0.05$). The variables pH, dry matter content, digestibility and metabolizable energy were affected by the interaction between harvest date and mixture ($p<0.05$). RT quality had less variation between HD1 and HD2 and BT had more CP, less fiber and presented higher digestibility and energy content ($p<0.05$).

Limitations on study/implications: Knowing the characteristics of a cereal mixture depending on the harvest date can help in making decisions to produce quality silage.

Findings/conclusions: Harvest date influences the proportion of components in small grain cereal mixtures for silage, on their morphological and nutritional composition; effect that depend to the cereal species in the mix.

Keywords. *Hordeum vulgare*; *Secale cereale*; *Triticosecale* Wittmack; forage mixture; silage.

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INTRODUCTION

Livestock systems may be affected in seasons when the availability of forages is limited or their quality is sub-optimal (Hackney *et al.*, 2021).

Conserved forage as silage is a strategy that overcomes feed deficiencies in these seasons, and to potencialize animal performance in critical periods (Mancipe-Muñoz *et al.*, 2020).

There is a recent interest in alternative forage crops, with a preference for small-grain cereals (SGC) for forage conservation (Hargreaves *et al.*, 2009). Among SGC there is barley (*Hordeum vulgare*), rye (*Secale cereale*), and triticale (*X. Triticosecale* Wittmack) (Payne *et al.*, 2008); which have been included in ruminant diets since they provide good quality fiber for adequate rumen function (Mancipe-Muñoz *et al.*, 2020).

These are sown as monoculture or as binary crops, with benefits of the latter in terms of productivity an quality (Liesch *et al.*, 2011). In that sense, silages from small-grain cereal forage mixtures have been demonstrated as an option to provide low cost forage for times of scarcity (Carrillo-Hernández *et al.*, 2023).

Harvest date after sowing and forage species are among the factors that may affect silage quality (Hargreaves *et al.*, 2009; Green *et al.*, 2014). Harvest date has an influence on the development cycle of the plants and on their phenological stage, which may negatively affect the leaf:stem ratio, with the ensuing decrease in nutritional quality of silage (Simionatto *et al.*, 2019).

Changes in the digestibility and chemical composition of a forage are due to a combination between morphological composition and plant growth (Elgersma y Søegaard, 2017). Previous reports showed that a delay in harvest date in SGC monocultures, due to incresead maturity, have increased fiber content and lower protein and digestibility (Hargreaves *et al.*, 2009, Coblentz *et al.*, 2018; Lyu *et al.*, 2018). However, there is no information on how this factor affects a SCG mixture, which may help in deciding the optimal timing for harvesting for silage.

There is differentiated development among SGC species, so that the optimal time of harvest also differs (Green *et al.*, 2014; Lyu *et al.*, 2018). It is recommended that triticale and rye are cut in boot or early milk stage, and barley harvested at the soft dought stage (Juskiw *et al.*, 2000). These may lead to a complementarity in their mixtures since rye and triticale have a slower growth than barley sot that it may be possible to a SGC mix to be on optimal cutting stage for all species depending on harvest date (Kaut, 2008); with a positive effect on the final quality of the mixture.

Therefore, the objective was to evaluate the effect of harvest date after sowing on the botanical, morphologic and nutritional composition of silage form mixtures of small-grain cereals.

MATERIALS AND METHODS

Study area

Crops were sown in the Central Highlands of Mexico in the municipality of Aculco (20° 12’ N y 99° 57’ W) with a mean altitude of 2400 m. Climate in the region is sub-humid temperate with a marked rainy season between May and October (800 mm rainfall) and a dry season between November and April, with a mean temperature of 13.2 °C (Gómez-Miranda *et al.*, 2023).

Laboratory silages and chemical analyses were undertaken at the Instituto de Ciencias Agropecuarias y Rurales (ICAR) of Universidad Autónoma del Estado de México.

Treatments and experimental design

Laboratory micro-silos were made from forage samples from three mixtures of small-grain cereal crops from barley – BLY (*Hordeum vulgare* cv. Cerro Prieto), rye (RYE) (*Secale cereale* cv. Criollo), and triticale (TRT) (*X. Triticosecale* Wittmack cv. Bicentenario) sown in two species mixtures:

BR=BLY+RYE; BT=BLY+TRT; and RT=RYE+TRT

These crops were cut in two harvest dates, 60 days post-sowing (HD1), and 80 days post-sowing (HD2).

The assessment followed a factorial 3×2 expetimental design, with the three crops and the two harvest dates as factors for a total of six treatements: BRHD1, BRHD2, BTHD1, BTHD2, RTHD1, RTHD1. Six silages (replicates) were made for each crop and harvest date (treatment) for a total 36 micro-silos.

Crops and silages

Each crop (binary SGC mixture) was sown in 1.0 h at a rate of 150 kg seed/ha (50% seed of each species), and fertilized with 100-60-60 NPK/ha.

Forage was cut by hand at a height of 10 cm above ground level, for ensiling, at 60 and 80 days post-sowing. In line with the scale proposed by Zadoks (1974), RYE and TRT were finalizing anthesis (Z68 to Z69), and BLY was at the early milk stage (Z72 to Z74); and at 80 days RYE and TRT were at the medium or late milk stage (Z75 to Z77), and BLY at the soft dough stage (Z85).

Cut forage was chopped to a 2.5 cm particle size and manually compacted in 40×10 cm polyethilene bags within PVC pipes of 50×10 cm with a 2.2 L capacity, following procedures described by Sainz-Ramírez *et al.* (2020).

Once fulled, the micro-silos were sealed and stored, and opened at 35 days. Martínez-Fernández *et al.* (2013) stated that after 20 days silage reaches stability, but for practical purposes the recommendation is to wait for a month before opening silos.

Variables

Three 200 g samples of each harvested forage before chopping were taken from each treatment for separation of botanical and morphological composition (stem, leaf, and spike for each species).

At silo opening, two sub-samples of silage were taken at three depths in each micro-silo (10, 20, and 30 cm). The first subsample was to record pH with a digital pH-meter (pH/mV/ °C meter OAKLON®), and the second subsample was used to determine dry matter (DM) content by drying at 58 °C in a draught oven, and later for analyses of chemical composition in terms of neutral detergent fiber (NDF), acid detergent fiber (ADF), crude protein content (CP) and *in vitro* dry matter digestibility (IVDMD) following standard procedures described by Celis-Álvarez *et al.* (2016). Lastly, estimated metabolizable energy content (eME) was following AFRC (1993).

Statistical analyses

Data were analysed with Minitab vs 19 (Minitab LLC, State College, PA, USA) applying a general lineal model of a 3×2 factorial experimental design with the model:

$$Y_{ijk} = \mu + r_i + M_{xj} + H_{dk} + Mix * HD_{jk} + e_{ijk}$$

Where Y_{ij}=response variable, μ=general mean, r_i=effect due to replicates (i=1,2,3,4,5,6), M_{xj}=effect due to mixture (j=1,2,3), H_{dk}=effect due to harvest date (k=1,2), $Mix*HD_{jk}$= effect due to the interaction between mixture and harvest date and e_{ijk}=residual error.

RESULTS AND DISCUSSION

Botanical and morphological composition

The harvest date had an effect on the total presence of cereal and other plant species, and in the proportion of small-grain cereals in each mixture (Table 1).

Table 1. Botanical composition.

	Small-Grain Cereal Mix (Mix)			Mean HD	SEM Mix	SEM HD	SEM Mix*HD
	BR	BT	RT				
Cereal (g kg^{-1} DM)							
HD1	856.5	656.7	801.1	771.4	56.7	70.1*	11.5^{NS}
HD2	720.3	528.6	550.0	599.6			
Mean Mix	788.4	592.6	675.5				
Other plants (g kg^{-1} DM)							
HD1	136.0	340.8	193.2	223.3	57.0	71.4*	11.4^{NS}
HD2	279.5	468.5	447.0	398.3			
Mean Mix	207.8	404.7	320.1				
Dead (g kg^{-1} DM)							
HD1	7.4	2.5	5.8	5.2	16.0^{NS}	41.5^{NS}	20.7^{NS}
HD2	0.0	2.9	3.2	2.0			
Mean Mix	3.7	2.7	4.5				
Species (%)							
HD1BLY	14.4	29.3	-	-	-	-	-
HD1RYE	71.3	-	60.0				
HD1TRT	-	36.3	20.1				
HD1Others	13.6	34.1	19.3				
HD2BLY	3.6	21.8	-				
HD2RYE	68.4	-	40.3				
HD2TRT	-	31.1	14.7				
HD2Others	28.0	46.9	44.7				

BR, BLY-barley+RYE; BT, BLY+TRT- triticale; RT, RYE+TRT. Mix, mixture; HD, harvest date; SEM, standard error of the mean. ^{NS} $p>0.05$; *$p<0.05$.

In HD2 the cereal content decreased 17% whilst the component of other species increased samewise ($p<0.05$); such that in BT and RT, other plant species represented 45% of total composition.

Both TRT and BLY had a weak initial development affecting the vigour of the crop, which favored the growth of unwanteD spontaneous vegetation even from the first harvest date (HD1), such that in BT cereal content was 20% less from the beginning ($p=0.06$).

Gómez-Miranda *et al.* (2022) attributed the growth of spontaneous vegetation to difficult agroclimatic and management conditions for small-grain cereal crops in the study area.

Gómez-Miranda *et al.* (2023) and Vega-García *et al.*, (2023) reported more than 60% content of spontaneous plants in monocrops of barley, rye and triticale for silage.

The proportion of different plant components in a forage mixture changes in time, being higher in the first cut (Klimek-Kopyra *et al.*, 2017). The evaluated cereal species reduced their proportion in HD2. BLY was the least present in the mixtures such that in BR its proportion in HD2 was 75% lower than in HD1, being dominated by RYE.

Zajac *et al.* (2014), evaluating cereal mixtures for grain production, reported that in a mixed RYE and BLY crop, RYE showed higher competitiveness, which affected the development of BLY. Klimek- Kopyra *et al.* (2017) stated that RYE has a strong competion ability, and shows dominance in binary mixtures sith other small-grain cereals, which explains its higher presence in this study.

There were morphological changes due to harvest date on each species due to effects of grwoth state on plant maturity (Table 2). In HD1, there was a 75% higher proportion of stems than leaves, and 59% higher than the proportion of spikes. This composition changed in HD2 when there was a higher proportion of spikes, except for RYE, with 55% in BLY and 42% in TRT; and lower values for the leaf compontne in the three cereal species (BLY, 11%; TRT, 4%; RYE, 3.5%).

The evaluated cereal species had completed their vegetative state by HD1, which explains the low leaf component compared to the other components Barón *et al.* (2015) stated that once flowering commences, the proportion of stem increases and leaf decreases, whilst the spike increases in weight as grain filling and maturity progresses, which explains the higher proportion of spikes in HD2.

Table 2. Mean values for morphological composition of small-grain cereals in two harvest dates (%).

Mix	Species	HD1			HD2		
		Stems	Leaves	Spikes	Stems	Leaves	Spikes
BR	BLY	40.4	30.8	28.9	33.3	11.1	55.6
	RYE	73.0	8.8	18.2	71.1	3.7	25.2
BT	BLY	52.7	18.8	28.6	33.8	11.5	54.7
	TRT	61.1	12.6	26.3	48.4	5.6	46.0
RT	RYE	72.2	9.0	18.7	78.1	3.4	18.5
	TRT	65.1	9.7	25.2	59.6	2.5	37.9

BR, BLY-barley+RYE; BT, BLY+TRT- triticale; RT, RYE+triticale- TRT. HD, harvest date.

Among species, BLY showed tha highest proportion of leaves and spikes, and lower stem proportion, followed by TRT, and lastly by RYE, which was the cereal that, independent of harvest date, sowed the mean lower proportion of leaves (6.2%) and spikes (20.2%) and a high proportion of stems (74%).

Neumann *et al.* (2019) stated thar RYE is characterized by a high proportion of stem, and BLY, given the lower size of plants, shows a lower content of stems and higher proportion of leaves, which favors its nutritional value. Also, BLY grows faster than RYE and TRT (Lyu *et al.*, 2018; Kaut *et al.*, 2008), which explains the higher proportion of spikes in BLY reported in Table 2.

Chemical composition of silages

Values for pH, DM, IVDMD and eME had a significant ($p<0.05$) interaction between cereal mix and harvest date (Table 3), but the interaction was not significant ($p>0.05$) for CP, NDF and ADF although there were significant effects for the main factors Mix and HD.

Muck *et al.* (2020) stated that a pH value around 4.0 enables a correct fermentation and silage stability, so values herein reported (between 3.8 and 4.3) were adequate.
As harvest date increased, pH incresed in HD by 7% for BR, and 12% for BT, with a mean increase of 85.5 g/kg in DM content in HD2 compared to HD1. Silages made from forages with high DM content have less available soluble carbohydrates (sugars), yielding loss fermentation products (organic acids) so that these silages have higher pH values (Muck *et al.*, 2020).

In general, pH is directly related to DM content of ensiled forages; the higher the DM content, the higher the pH value (Simionatto *et al.*, 2019); which explanis the lower pH values for the BT mix, as it had the lower DM content ($p<0.05$).

Mean DM content was 262 g/kg (202.7 to 346.5 g/kg), under the optimal range between (300 - 400 g/kg^{-1} DM) put forward by Muck *et al.* (2020). Nonetheless, DM content did not affect a correct fermentation in the silages as the pH values demonstrated.

DM content of silages increased between HD1 and HD2 as forages were more mature. However, that increase was in a differente proportion among the evaluated cereal mixes as shown by the significant interaction between Mix and HD.

The treatments that included TRT increased a mean of 22% in their DM content, while the BR Mix increased DM content in 32% due to the dominance of RYE, the cereal with a high proportion of stems, which have a higher DM content than the leaf or spike component.

Mean CP content decreased 29% (31 g/kg^{-1} DM) between HD1 and HD2 ($p<0.05$); while NDF and ADF increased 6.0% and 9.6% respectively ($p<0.05$), except for RT that jad constant fiber values between HD1 and HD2.

Observed differences are due to the normal maturation process in cereals, which brings about higher concentrations of fiber and reductions in CP content as the harvest date is delayed (Coblentz *et al.*, 2018).

Hargreaves *et al.* (2009) reported low CP values for immature BLY forage (100 g/kg^{-1} DM) that progressively decreased to less than 70 g/kg^{-1} DM in more mature forage; a value similar to findings in the experiment herein reported.

Table 3. Chemical composition of silage from mixed small-grain cereals in two harvest dates.

	Mixture (Mix)			**Mean HD**	**SEM Mix**	**SEM HD**	**SEM Mix*HD**
	BR	**BT**	**RT**				
pH							
HD1	4.1	3.8	4.3	4.1	0.1*	0.1*	0.0*
HD2	4.4	4.3	4.3	4.3			
Mean Mix	4.3[a]	4.1[b]	4.3[a]				
DM (g kg^{-1})							
HD1	234.3	202.7	232.9	223.3	29.3*	54.3*	9.0*
HD2	346.9	261.2	292.2	300.1			
Mean mx	290.6[a]	232.0[c]	262.5[b]				
CP (g kg^{-1} DM)							
HD1	105.6	112.0	100.9	106.2	4.8*	21.9*	0.8[NS]
HD2	72.3	80.2	73.2	75.2			
Mean mx	89.0[ab]	96.1[a]	87.1[b]				
NDF (g kg^{-1} DM)							
HD1	610.5	505.9	610.5	575.6	58.1*	18.3*	6.7[NS]
HD2	654.6	539.7	610.3	601.6			
Mean mx	632.6[a]	522.8[b]	610.4[a]				
ADF (g kg^{-1} DM)							
HD1	212.7	176.0	207.6	198.7	29.9*	25.7*	3.5[NS]
HD2	233.3	204.0	221.6	219.6			
Mean pm	223.0[a]	189.9[c]	214.6[b]				
IVDMD (g kg^{-1} DM)							
HD1	584.8	710.2	566.5	620.5	72.8*	48.5*	11.6*
HD2	484.2	628.7	542.9	551.9			
Mean mx	534.5[b]	669.4[a]	554.7[b]				
eME (MJ kg^{-1} DM)							
HD1	8.1	9.9	7.9	8.7	10.5*	7.0*	0.2*
HD2	6.7	8.8	7.5	7.7			
Mean mx	7.4[b]	9.4[a]	7.7[b]				

BR, BLY-barley+RYE; BT, BLY+TRT-triticale; RT, RYE+TRT. DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; IVDMD, *in vitro* dry matter disgestibility; eME, estimated metabolizable energy. SEM, standard error of the mean. [NS] $p>0.05$; *$p<0.05$.

Similarly, Geren (2014) informed that the mean crude protein content for SGC also evaluated in the present work decreased from 118 g/kg DM (at early heading) to 89 g/kg^{-1} DM (in mid-dough stage), and the NDF content increased from 494 g/kg^{-1} DM to 607 g/kg^{-1} DM.

Changes in the botanical and morphological components in the cereal mixtures on the harvest dates affected the nutritional value of each silage. Mix BT had higher CP and lower NDF and ADF compared BR and RT ($p<0.05$), and treatments with RYE did not show differences for these variables ($p>0.05$).

The individual components of the BT treatment (BLY and TRT) are reported in the literature with highr CP content and less NDF and ADF compared to RYE (Geren, 2014); the species that dominated botanical composition in the BR and RT treatments, given its stem proportion of over 70%. Stem content is related to increased NDF and a decrease in Nitrogen content due to a thicker cell wall and lower soluble cell contents (Elgersma and Søegaard, 2017).

Fiber contents are inversely related to digestibility of forages (Elgersma and Søegaard, 2017), as observed in Table 3 where as NDF increased between HD1 and HD2, there was a decrease in IVDMD and consequently in eME content; although the magnitude was dependant on the cereal species in each treatment.

As the significant interaction between Mix and HD showed, IVDMD in RT decreased 4% between HD1 and HD2; while the decrease in IVDMD for BT and BR was 11.3% and 17.4% respectively. That was due because the botanical components in RT (RYE and TRT) have lower variation in their nutritional value between phenological stages (Lyu *et al.*, 2018; Simionatto *et al.*, 2019). This feature enabled harvesting up to 20 days after completion of the anthesis stage, without loss in digestibility, an important factor when due to climatic conditions or other factors it is necessary to delay ensiling.

On the other had, the reduction in IVDMD in BT between HD1 and HD2 could have been due to the fact that BLY reaches maturity faster than TRT and likewise, it nutritive value decreases more rapidly (Lyu *et al.*, 2018).

Lastly, BR was more affected by the harvest date due to the decreased proportion of BLY in HD2 and the dominance of RYE.

BT had an IVDMD and eME 17% and 25% higher compared to BR and RT; since the RYE in this treatment reduced their digestibility due to the high proportion of stems, while BT had a better ratio between morphological components, whith higher proportion of leaves and spikes that favored its digestibility.

Since stems are support structures, they contain more lignified tissues than other morphological components, and therefore, have a lower digestibility (Moore *et al.*, 2020); while spikes in SGC increase their nutritional value due to the formation and fill-up of grain increasing starch content and therefore, their digestibility (Baron *et al.*, 2015).

There is scarce information on the nutritional value of silages from SGC mixtures. Carrillo Hernández *et al.* (2023) reported for a mixture of BLY and RYE at the milk stage, with RYE dominance, and IVDMD of 538.9 g/kg^{-1} DM.

Coblentz *et al.* (2018) working with different TRT cultivars in monoculture reported digestibilities of 610 g/kg^{-1} DM at anthesis, and 606 g/kg^{-1} DM in milk stage.

Lyu *et al.* (2018) reported for BLY and TRT in anthesis an IVDMD of 681.0 and 648.9 g/kg^{-1} DM, and in soft doughn 659.6 g/kg^{-1} DM for BLY and 636.1 g/kg^{-1} DM for TRT, similar values to those herein reported for the mix of these two species.

Small-grain cereals do not develop at the same rhythm or with the same patterns, so that each species reach its optimal harvest stage in different times (Lyu *et al.*, 2018). Therefore, there is the need to ensure the best quality and adequate DM content for ensiling at harvest of a SGC mix, avoiding the grouth of undesireble spontaneous vegetation.

Ensiling can be between 67 and 70 days post-sowing, the time for RYE and TRT to reach the early milk stage (Z73-Z75) and BLY would reach early dough (Z81 a Z83) in the Zadoks (1974) scale. These phenological stages are adequate for ensiling small-grain cereal forages.

CONCLUSIONS

Harvest date has an effect on the proportion of components in small-grain cereal mixtures for silage, in their morphologic and nutritional composition. The effects are due to the cereal species in the mix, being the rye and triticale (RT) treatment the most stable across the harvest dates; and the barley and triticale (BT) treatment the mix with the higher nutritional value.

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REFERENCES

Hackney, B., Rodham, C., Dyce, G., & Piltz, J. (2021). Pasture legumes differ in herbage production and quality throughout spring, impacting their potential role in fodder conservation and animal production. *Grass Forage Sci*. 76, 116–133. Doi: 10.1111/gfs.12525

Mancipe-Muñoz, E., Vásquez-Vanegas, J.E., Castillo-Sierra, J., Ortiz-Cuadros, R.E., Avellaneda-Avellaneda, Y., & Vargas-Martínez, J.D.J. (2021). Productividad y valor nutricional de forraje de cebada y trigo del trópico alto colombiano. *Agron. Mesoam. 32*(1), 270-291. DOI: 10.15517/am.v32i1.40465

Hargreaves A., Hill J., & Leaver, J. D. (2009). Effect of stage of growth on the chemical composition, nutritive value and ensilability of whole-crop barley. *Anim. Feed Sci. Technol. 152*(1-2), 50–61. Doi: 10.1016/j.anifeedsci.2009.03.007

Payne T.S., Amri A., Humeid B., & Rukhkyan N. (2008). Guías para la regeneración de germoplasma: cereales de grano pequeño. En: Dulloo M.E., Thormann I., Jorge M.A. and Hanson J., editors. Crop specific regeneration guidelines [CD-ROM]. CGIAR System-wide Genetic Resource Programme (SGRP), Rome, Italy. 12 pp.

Liesch, A.M, Krueger, E.S., & Ochsner, T.E. (2011). Soil Structure and Physical Properties under Rye-Corn Silage Double-Cropping Systems. *Soil Sci. Soc. Am. J. 75*(4), 1307-1314. Doi: 10.2136/sssaj2010.0292

Carrillo-Hernández S., Velarde-Guillén J., López-González F., & Arriaga-Jordán CM. (2023). Ensilados mixtos de cereales de grano pequeño en la alimentación de vacas en sistemas de producción de leche en pequeña escala. *Trop. Subtrop. Agroecosyt. 26*(85).

Geren, H. (2014). Dry matter yield and silage quality of some winter cereals harvested at different stages under mediterranean climate conditions. *Turk. J. Field Crops. 19*(2), 197-202. Doi: 10.17557/tjfc.55884

Simionatto, M., Maeda, E. M., Fluck, A. C., Silveira, A. P., Piran, F. A., Paula, F. L. M., & de Macedo, V. de P. (2019). Nutritional and morphostructural characterization of pre-dried winter grass silage. *Semina: Ciênc. Agrár. 40*(5), pp. 2375-2386. Doi: 10.5433/1679-0359.2019v40n5Supl1p2375

Elgersma, A., & Søegaard, K. (2017). Changes in nutritive value and herbage yield during extended growth intervals in grass-legume mixtures: effects of species, maturity at harvest, and relationships between productivity and components of feed quality. *Grass Forage Sci. 73*(1), 78–93. Doi: 10.1111/gfs.12287

Coblentz, W-K., Akins, M.S., Kalscheur, K.F., Brink, G.E., & Cavadini, J.S. (2018). Effects of growth stage and growing degree day accumulations on triticale forages: 1. dry matter yield, nutritive value, and *in vitro* dry matter disappearance. *J. Dairy Sci. 101*(10),8965–8985 DOI: 10.3168/jds.2018-14868

Lyu, D., Doce, R.R., Juskiw, P., Zhou, G., & Baron, V. S. (2018). Diverse grain-filling dynamics affect harvest management of forage barley and triticale cultivars. *Agron. J.* 110,1017–1027.Doi: 10.2134/agronj2017.09.0549

Juskiw, P.E., Helm, J.H., & Salmon, D.F. (2000). Postheading biomass distribution for monocrops and mixtures of small grain cereals. *Crop Sci.* 40, 148–158.

Kaut, A.H.E.E., Mason, H.E., Navabi, A., O'Donovan, J.T., & Spaner, D. (2008). Organic and conventional management of mixtures of wheat and spring cereals. *Agron. Sustain. Dev.* 28, 363–371. Doi: 10.1051/agro:2008017

Gómez-Miranda, A., Arriaga-Jordán, C.M., Vieyra-Alberto, R., Castro-Montoya, J.M., & López-González, F. (2023). Evaluation of weed silage in dairy cattle feeding in small-scale dairy systems. *Trop. Subtrop. Agroecosyt, 26* (074).

Zadoks, J.C.T., Chang, T.T., & Konzak, C. (1974). A decimal code for the growth stages of cereals. *Weed Res.* 14, pp. 415–421. Doi:10.1111/j.1365-3180.1974.tb01084.x

Sainz-Ramírez, A., Botana, A., Pereira-Crespo, S., González-González, L., Veiga, M., César,R., Valladares, J., Arriaga-Jordán, C.M., & Flores-Calvete G. (2020). Efecto de la fecha de corte y del uso de aditivos en la composición química y calidad fermentativa de ensilado de girasol. *Rev Mex Cienc Pecu, 1*(3):620-637. Doi: 10.22319/rmcp.v11i3.5092

Celis-Álvarez, M.D., López-González, F., Martínez-García, C.G., Estrada-Flores, J.G., & Arriaga- Jordán, C.M. (2016). Oat and ryegrass silage for small-scale dairy systems in the highlands of central Mexico. *Trop. Anim. Health Prod.* 48, 1129–1134. Doi: 10.1007/s11250-016-1063-0

Martinez-Fernández, A., Soldado, A., Roza-Delgado, B., Vicente, F., Gonzalez-Arrojo, M.A., & Gonzalez-Arrojo, A (2013) Modelling a quantitative ensilability index adapted to forages from wet temperate areas. *Span. J. Agric. Res.* 11, 455-462.

AFRC. 1993. Energy and protein requirements of ruminants. Agricultural and Food Research Council. CAB International, Wallingford, UK.

Gómez-Miranda, A., López-González, F., Vieyra-Alberto, R., & Arriaga-Jordán, C.M. (2022). Grazed barley for dairy cows in small-scale systems in the highlands of Mexico. *Ital. J. Anim. Sci. 21*(1), 178–187. Doi: 10.1080/1828051X.2021.2022540

Vega-García, J.I, López-González, F, Morales-Almaraz, E., & Arriaga-Jordán, C.M. (2022). Secondary growth rye or triticale silage: Small-grain cereals as a dual-purpose forage option for small-scale dairy systems in the highlands of Mexico. *Chil. J. Agric. Res. 83*(1). Doi: 10.4067/S0718-58392023000100031

Klimek-Kopyra, A., Bacior, M., & Zajac, T. (2017). Biodiversity as a creator of productivity and interspecific competitiveness of winter cereal species in mixed cropping. *Ecol. Model.* 343, pp. 123–130. Doi: 10.1016/j.ecolmodel.2016.10.012

Zajac, T., Andrzej, O.A., Stokłosa, A., Klimek-Kopyra, A. Styrc, N., Mazurek, R., & Budzynski, W. (2014). Pure sowings versus mixtures of winter cereal species as an effective option for fodder–grain production in temperate zone. *Field Crops Res.* 166, 152–161. Doi: 10.1016/j.fcr.2014.06.019

Neumann, M., Dochwat, A., Horst, E.H., Venancio, B.J., Santos, J.C., Heker, J.C., Braga, F., Santos, L.C., & Silva, E.P. (2019). Productivity, profitability and nutritional quality of forage and silage of winter cereals. *Semina: Ciênc. Agrár. 40*(3), 1275. Doi: 10.5433/1679-0359.2019v40n3p1275

Muck, R.E., Kung, Jr., & Collins Michael, L. (2020). Silage production,42. In Forages: The Science of Grassland Agriculture 7th Edn. K.J. Moore, M. Collins, C.J. Nelson, and D.D. Redfearn, Eds. John Wiley & Sons Ltd, Ames, IA, USA, 2020. Volume II Pág. 767-787. Doi: 10.1002/9781119436669.ch39

Moore, J.K., Curtiss C.F., Lenssen, A.W., & Fales, S.L. (2020). Factors Affecting Forage Quality 39. In Forages: The Science of Grassland Agriculture 7th Edn, K.J. Moore, M. Collins, C.J. Nelson, and D.D. Redfearn, Eds. John Wiley & Sons Ltd, Ames, IA, USA, 2020; Volumen 2, pág. 701-716. Doi: 10.1002/9781119436669.ch39

Baron, V.S., Juskiw, P.E., & Aljarrah, M. (2015). Triticale as a Forage. In Triticale. F. Eudes, eds. Springer Int. Publishing, New York, USA, 2015; pág. 189-212. Doi: 10.1007/978-3-319-22551-7_10

Colegio de Postgraduados

Determination of bioactive compounds and physicochemical parameters of honey produced in the state of Veracruz, Mexico

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ABSTRACT

Veracruz occupies one of the first places as a producer of honey. However, there are few studies that address the typing of bioactive compounds, and the physicochemical characteristics present in Veracruz honey.

Objective: Determine the physicochemical and antioxidant parameters, and total phenols (TP) of Veracruz honey.

Design/methodology/approach: Honey samples were collected at 17 sites in the state of Veracruz, and physicochemical, TP and antioxidant parameters were analyzed.

Results: The determined physicochemical parameters presented values within the ranges set by NOM-004-SAG/GAN-2018 and the *Codex Alimentarius*. The color distribution showed the following values: dark (47.6%), amber (19.04%), white (19.04%), and the colors aqua white, light amber and extra light showed values of 4.77% each. Dark honeys presented ~370 μg GAE/mL, compared to the contents shown by light-colored honeys of ~200 μg GAE/mL. Sayula de Aleman honey had the highest antioxidant content with 143 μg TE/g honey. On the other hand, honey from San Pedro Soteapan showed the lowest contents (53 μg TE/g honey).

Limitations on study/implications: The selection of Veracruz honey apiaries and lack of flora information.

Findings/conclusions: The honeys presented physicochemical parameters within ranges of national standards. These Veracruz honeys exhibited a range of colors from dark to extra light. A positive correlation was shown between color and TP content. The antioxidant content was dependent on the botanical origin and color of these honeys.

Keywords: honey color, phenolic compounds, antioxidants.

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INTRODUCTION

Bee honey by definition is a sweet natural compound, which is produced thanks to the participation of an insect commonly known as "bee", which includes several species such as: *Apis mellifera*, *Melipona beecheii*, among others. The bee collects nectar from flowers,

secretions or living parts of the plant, which can be combined with different substances from its saliva, so these are transformed and then stored in the honeycombs of the hive, where the chemical transformation culminates to become honey (*Codex Alimentarius*, 2019). This process has been identified as usually occurring in two parts: the first involves evaporation, where this nectar loses an average of a third of its moisture during storage. In the next step, the enzyme invertase hydrolyzes sucrose to produce the monosaccharides fructose and glucose (Lichtenberg-Kraag, 2014; Nicolson *et al.*, 2022; Alaerjani *et al.*, 2022).

Honey contains approximately ∼180 different molecules ranging from carbohydrates, water, organic acids, enzymes, amino acids, pigments, pollen, among others. Therefore, this gives honey a high nutritional value, in addition to attributing various bioactivity properties to it. Some studies have revealed the antioxidant effects of honey, coupled with its antimicrobial, anti-inflammatory and anti-proliferative properties (Samarghandian *et al.*, 2017; Silva *et al.*, 2021). Particularly, antioxidants are compounds that can prevent and/or inhibit free radicals, which are responsible for oxidative stress in humans and cause various diseases. It has been reported that these antioxidants could correspond to phenolic compounds, flavonoids, ascorbic acid, catalase, peroxidase, alkaloids, amino acids and chlorophyll derivatives, among others. Among the antioxidants detected in honey are α-tocopherol, carotenoids, proteins and melanoidins (Brudzynski & Miotto, 2011; Al-Farsi *et al.*, 2018). It has been proposed that the chemical composition of honey is a function of several factors, such as the flowers that the bee visits to collect nectar, beekeeping management, storage, soil chemistry, geographical conditions, and even the climatic conditions.

It is worth mentioning that for the world market, Mexican honey has great acceptance and demand due to its quality, nutritional value, pleasant sensory characteristics, and color. According to statistics, Mexico occupies ninth place as a honey producer and thirteenth as the largest exporter, with a value of ∼118.6 million dollars, with the United States, the European market (Germany, Netherlands, France, the United Kingdom, Switzerland, Belgium and Spain), Saudi Arabia and Japan as its main clients (SIAP, 2022). The main honey producing states are Yucatán, Campeche, Jalisco, Chiapas, Veracruz, Oaxaca, Quintana Roo, Puebla, Michoacán, Guerrero, Zacatecas, Morelos, Hidalgo and San Luis Potosí. The state of Veracruz occupies 5th place as one of the main honey producers in the country (SIAP, 2022).

This beekeeping activity, both in the country and in the state, is facing several problems, among which we can mention the attack of pests, particularly the Varroa destructor mite, climate change, which in recent years has caused long periods of drought, lack of infrastructure in the honey production chain, and/or an increase in the market for honey adulterated with fructose; all these factors have strongly impacted the productivity and profitability of beekeeping activity (Magaña-Magaña *et al.*, 2016). All of the above has caused the majority of apiaries to fail to standardize the quality of honey, which is why a differential number of batches arrive on the market that do not always meet the specifications required and established by the Mexican Standard (NOM-004-SAG/GAN-2018). Beekeeping in the state of Veracruz requires, in the medium term, to strengthen the honey production chain. For this, among its first actions, it is necessary to carry out work aimed at complying with the quality parameters that allow it to obtain certifications and compete in national and international markets.

Therefore, it is necessary to carry out basic research focused on establishing the bases to classify the physicochemical quality, the content of phenolic compounds, as well as the antioxidant compounds present in honey produced in the state of Veracruz.

MATERIALS AND METHODS

The honey samples were provided by producers from 17 different sites in the state of Veracruz (north, center and south); Soconusco (17° 57' 47"N - 94° 52' 50" W), Texistepec (17° 53' 43" N - 94° 49' 01" W), San Pedro Soteapan (18° 13' 48" N - 94° 52' 19" W), Cuatotolapan, Hueyapan de Ocampo (18° 08' 46" N - 95° 17' 59" W), Playa Vicente (17° 49' 48" N - 95° 48' 48" W), Villa Juanita (17° 48' 32" N - 95° 13' 11" W), San Andrés, Tuxtla (18° 26' 18" N - 95° 12' 45" W), Almagres (17° 48' 25" N - 94° 55' 02" W), Valle del Uxpanapa (17° 14' 18" N - 94° 26' 21" W), Sayula de Alemán (17° 52' 47" N - 94° 57' 30" W), Comején (18° 03' 39" N - 94° 53' 03" W), Álamo Temapache (20° 54' 22" N - 97° 41' 24" W), Hueyapan de Ocampo (18° 08' 58" N - 95° 08' 38" W), Colmena, Soconusco (17° 59' 36" N - 94° 50' 31" W), Aguilera, Sayula de Alemán (17° 48' 40" N - 95° 00' 58" W), Coatepec (19° 26' 59" N - 96° 57' 31" W), Chinameca (18° 01' 11" N - 94° 40' 44" W). Consequently, the northern zone has a semiwarm subhumid climate with sub-deciduous forest and palm grove vegetation, while the center of the state has a temperate climate with mesophilic mountain forest vegetation, and in the southeast of the state, the climate is semiwarm and humid with tropical forest vegetation, grasslands, and secondary vegetation (INEGI, 2020). These honey samples with characteristic aroma, flavor and without signs of fermentation. These were compared with two honey samples from two commercial brands: Carlota® and Vita Real®.

To determine the pH and total acidity, a HANNA 211® potentiometer was used, according to the Mexican Standard (NOM-004-SAG/GAN-2018). A portable dual ATC® refractometer was used to determine, according to the Mexican Standard (NOM-004-SAG/GAN-2018), the content of soluble solids and humidity of the collected honey samples (g/100 g honey).

The color of the honey was determined by spectrophotometry (BioRad SmartSpec Plus®; Codex Alimentarius, 2019). The readings were made at an absorbance of 560 nm and according to the Pfund scale. The extraction of total phenols (TP) was carried out using the protocol published by Elvia *et al.*, 2015, to which some modifications were made. The analysis of TPs was performed spectrophotometrically at 760 nm using the Folin-Ciocalteu method, reported by Singleton *et al.*, 1999 with some modifications. The antioxidant capacity was estimated using ABTS•+(2,2'-zino-bis(3-ethylbenzothiazolin)-6-sulfonic acid, A-1888), potassium persulfate ($K_2S_2O_8$) and as standard Trolox (6-hydroxy acid -2,5,7,8-tetramethylchromium-2-carboxylic acid 97%) as reference oxidizing agent. The antioxidant activity was expressed as grams of Trolox Equivalent (TE) per gram of honey (g TE/g honey) according to what was reported by Re *et al.*, 1999, with modifications. Assays were performed in triplicate and the results were expressed as the mean values with standard deviations (±). The significant differences represented by letters were obtained by a one-way analysis of variance (ANOVA) followed by Tukey's ($P<0.05$) and Pearson's correlation analysis to obtain the r-value with the Minitab® 17 software.

RESULTS AND DISCUSSION

The results show that honey is a food of acidic nature, having a pH range of 3.28±0.14 to 4.42±0.23, and total acidity in a range of 20±1.45 to 35±2.75. These data coincide with what was reported by (*Codex Alimentarius*, 1981; NOM-004-SAG/GAN-2018; Pauliuc & Oroian, 2020; García-Chaviano *et al.*, 2022; Table 1). Furthermore, due to the pH ranges shown in this study, it is suggested that they could be honeys with floral origins because they are within the reported pH range of Cervera & Cervera, 1994, that was between 3.4 to 4.6. The total acidity was also evaluated, which is formed by the hydroxylated acids in honey, that is, they can be both acids and alcohols (Cervera & Cervera, 1994). The honey samples analyzed present values between 20±1.45 to 35±2.75 meq/Kg, which correspond to the limits marked by the standards (*Codex Alimentarius*, 1981; NOM-004-SAG/GAN-2018). It has been proposed that these acidity ranges protect honey from pathogenic microbial growth and, in turn, may contribute to aroma. Regarding soluble solids, honey samples collected in the state presented ranges from 76±0.0 to 89.6±0.0 °Brix. These values are similar and are in the same range as those already reported (Castillo-Martínez *et al.*, 2022), with values between 77 and 86 °Brix for honey from *Apis mellifera* and *Melipona beecheii*. On the other hand, the water content present in honey should not be greater than 23%, because it is very likely that honey will ferment, change its smell, flavor, and/or tend to crystallize. The humidity range presented by the honeys analyzed in this study was from 14.0 to 20.6%, values that are within the permissible limits (Table 1), being, therefore, another good indication of the maturity of these honey samples from Veracruz (Makhloufi *et al.*, 2010).

In the case of the color of the honey samples, the results were grouped by colors, where dark tones predominated, with an average of 47.6%, followed by the amber color with 19.04%, the white color with 19.04%, for the light amber only 4.77%, similar to aqua white with 4.77%, and finally extra light amber with the remaining 4.77%. This diversity in the types of honey, where they differ in both color and flavor, is perhaps due to the floral origin of each of them (Muñoz-Jáuregui *et al.*, 2014; Ávila *et al.*, 2019; Becerril-Sánchez *et al.*, 2021; Figure 1), due to the diversity that each of the honey collection areas present and its vegetation. In this sense, for example, Alamo Temapache honey, whose color is extra light amber, also presents notes of citrus flavors, this is since it is found among *Citrus* plantations in the region.

It has been proposed that the color of honey is due to pigments in the nectar of flowers and other parts of plants (Becerril-Sánchez *et al.*, 2021). Phenolic compounds are the main factor that can give the color and flavor properties of honey.

It is worth mentioning that it has been reported that dark honeys tend to be the richest in the content of minerals (Na, K, Ca, Mg, Fe, Cu, Zn, Al, Ni, Cd and Mn) and vitamins (B and C), while light-colored honeys have a higher content of vitamin A (Solayman *et al.*, 2016, Hungerford *et al.*, 2020).

Table 1. Physicochemical properties determined from honey samples collected in the different regions of the state of Veracruz.

Collection sites (and code)	pH	Total acidity (meq/kg)	°Brix	% Moisture
Soconusco (S)	4.42 ± 0.23^{a}	31 ± 1.80^{cde}	88 ± 0.11^{bc}	16 ± 2.0^{abcde}
Texistepec (T)	4.18 ± 0.15^{abcd}	33 ± 5.03^{a}	89.6 ± 0.00^{d}	15 ± 1.0^{de}
San Pedro Soteapan (SPS)	4.07 ± 0.06^{abcd}	$30\pm.50^{fgh}$	82.6 ± 0.30^{bcd}	15.5 ± 1.4^{bcd}
Cuatotolapan, Hueyapan de Ocampo (CHO)	3.28 ± 0.14^{h}	$26\pm.36^{cdef}$	78 ± 0.11^{d}	20.3 ± 0.9^{ab}
Playa Vicente (PV)	3.82 ± 0.40^{bcdef}	$22\pm.55^{fghij}$	85.3 ± 0.21^{cd}	14.6 ± 1.8^{de}
Villa Juanita (VJ)	4.01 ± 0.17^{abcd}	30 ± 2.225^{ghij}	80 ± 0.11^{d}	18.5 ± 0.5^{abcde}
San Andrés Tuxtla (SAT)	3.90 ± 0.08^{bcdef}	31 ± 1.04^{bc}	81 ± 0.20^{d}	17.4 ± 1.5^{abcde}
Almagres (ASA)	3.60 ± 0.11^{fg}	$28\pm.43^{cde}$	78.6 ± 0.1^{bcd}	20 ± 3.0^{abc}
Valle de Uxpanapa (VU)	3.81 ± 0.05^{bcdef}	23 ± 1.2^{cdefg}	80.7 ± 0.11^{bcd}	17.8 ± 0.2^{abcde}
Sayula de Aleman (SA)	3.91 ± 0.06^{abcde}	34 ± 4.07^{c}	82 ± 0.35^{bcd}	16.4 ± 1.4^{abcde}
Comején (C)	3.57 ± 0.07^{ef}	20 ± 1.451^{cde}	79.3 ± 0.28^{bcd}	19.5 ± 1.5^{abcd}
Álamo, Temapache (AT)	4.32 ± 0.18^{ab}	28 ± 0.32^{b}	86 ± 0.00^{bc}	14 ± 0.8^{e}
Hueyapan de Ocampo (HO)	3.88 ± 0.07^{bcdef}	$30\pm.52^{cdef}$	76 ± 0.00^{a}	20 ± 3.0^{abc}
Colmena, Soconusco (CS)	4.17 ± 0.12^{abcd}	27 ± 2.6^{cd}	81.3 ± 0.00^{b}	17.5 ± 2.0^{abcde}
Aguilera (AGSA)	3.85 ± 0.09^{cdef}	30 ± 0.52^{ef}	80 ± 0.34^{cd}	18.4 ± 1.4^{abcde}
Coatepec (CO)	3.80 ± 0.11^{def}	35 ± 2.75^{efgh}	83.3 ± 0.41^{bcd}	15.2 ± 2.0^{cde}
Chinameca (CHI)	4.32 ± 0.16^{abc}	$29\pm.1.2^{defg}$	82 ± 0.23^{ab}	16.4 ± 1.4^{abcde}
Carlota (CA)®	3.85 ± 0.02^{bcdef}	26 ± 0.75^{cdefg}	78 ± 0.57^{b}	20.6 ± 1.3^{a}
Vita Real (VI)®	3.90 ± 0.74^{bcdef}	25 ± 1.29^{fghij}	81.3 ± 0.00^{bcd}	17.1 ± 0.3^{abcde}

* Note: Tukey's analysis: Means that do not share a letter are significantly different (one-way ANOVA, $p<0.05$).

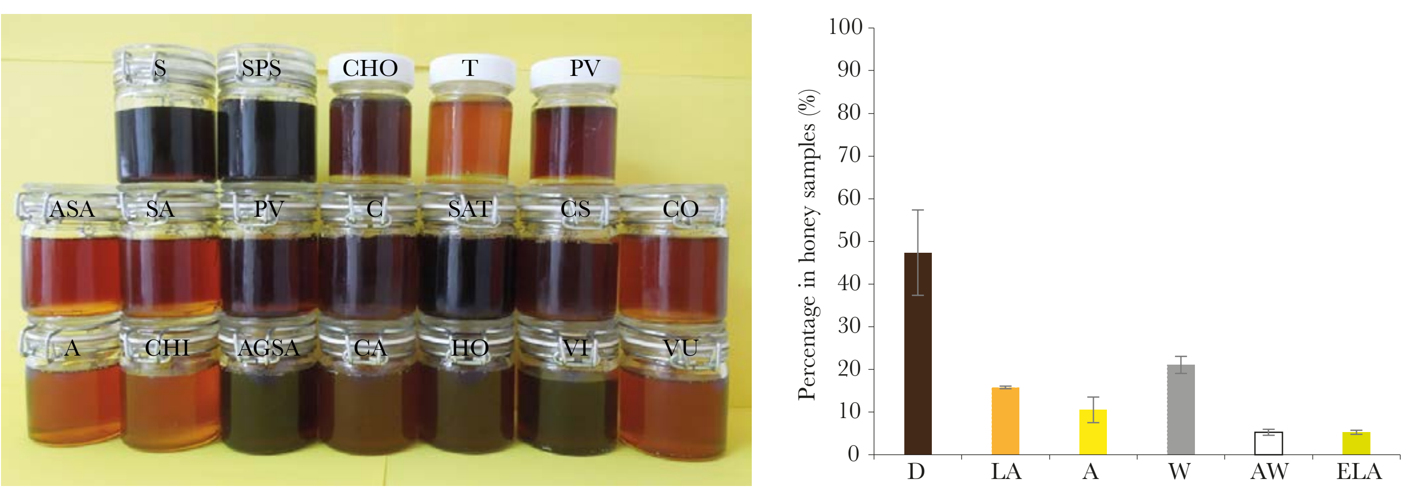

Figure 1. Classification of the color variation of honey samples collected from the different regions of the state of Veracruz. a) Color of honey samples, b) Color classification by the spectrophotometry method according to the Pfund scale. (D; Dark, LA; Light Amber, A; Amber, W; White, AW; Aqua White and ELA; Extra Light Amber).

The determination of the total phenol contents in the Veracruz honeys was carried out, and the results show approximate ranges from 200 to 370 GAE (μg gallic acid equivalent / mL) (Figure 2). The honey with the highest phenol content was the collected in Soconusco (S), with approximately 370 GAE (μg gallic acid equivalent/mL), while the lowest phenol content was found in the honey collected in the communities of Texistepec and Almagres, municipality of Sayula de Aleman, with 200∼204 GAE (μg gallic acid equivalent/mL).

When comparing these results, a positive correlation (r=0.54) is shown with the color of honey, that is, the higher the phenol content, the darker the honey, and the lower the phenol content of the honey, the lighter the color.

In this sense, having honeys with high phenolic compounds can be a strong indication of the presence of a high antioxidant capacity, which results in health benefits and can even reduce cellular stress and, therefore, delay aging in these organisms (Aljadi & Kamaruddin, 2004; Rodríguez *et al.*, 2012).

These phenolic compounds contained in honey are directly related and attributed to the bioactive properties of honey (Aljadi & Kamaruddin, 2004, Becerril-Sánchez *et al.*, 2021). Consequently, it is usually one of the characteristics that is important to market and provide added value to honey. In this sense, the price of honey for the international market depends largely on its color, flavor, and moisture content (Ciappini *et al.*, 2013).

Another of the important compounds in honey are antioxidants, considered as heterogeneous substances that are made up of vitamins (A, E and C), minerals, natural

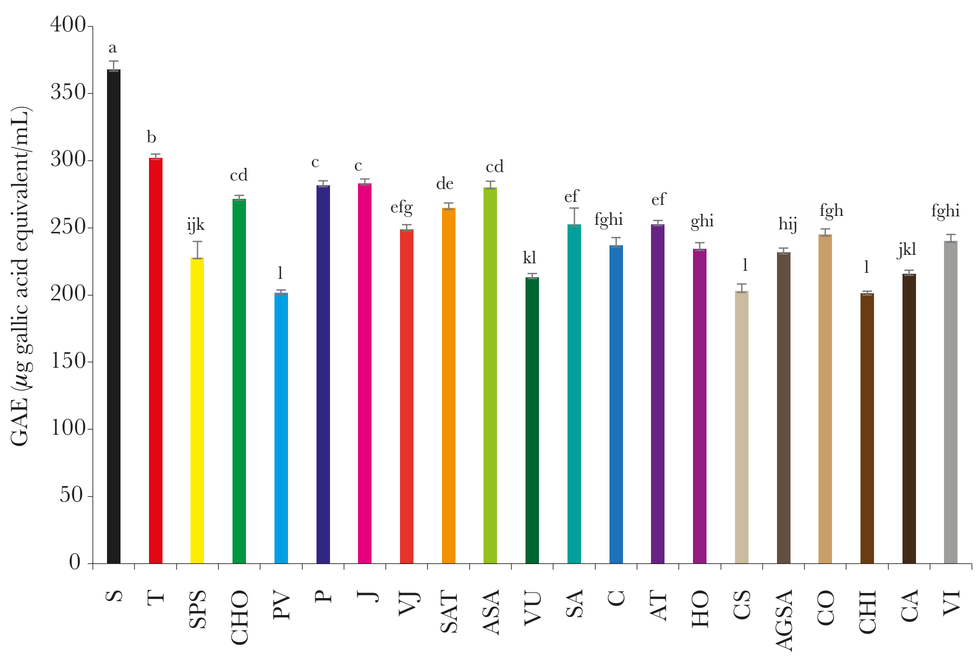

Figure 2. Determination of the total phenolic content in honey samples collected in the different regions of the state of Veracruz. Values are presented as mean ± standard deviation; furthermore, different letters in the columns indicate statistically significant differences (one-way ANOVA, $p<0.05$). (S) Soconusco, (T) Texistepec, (SPS), San Pedro Soteapan, (CHO), Cuatotolapan, Hueyapan de Ocampo, (PV), Playa Vicente, (VJ) Villa Juanita, (SAT) San Andrés Tuxtla, (ASA) Almagres, (VU) Valle de Uxpanapa, (SA) Sayula de Alemán, (C), Comejen, (AT), Álamo Temapache, (HO) Hueyapan de Ocampo, (CS) Colmena, Soconusco, (AGSA) Aguilera, (CO) Coatepec, (CHI) Chinameca, (CA) Carlota®, (VI) Vital Real®.

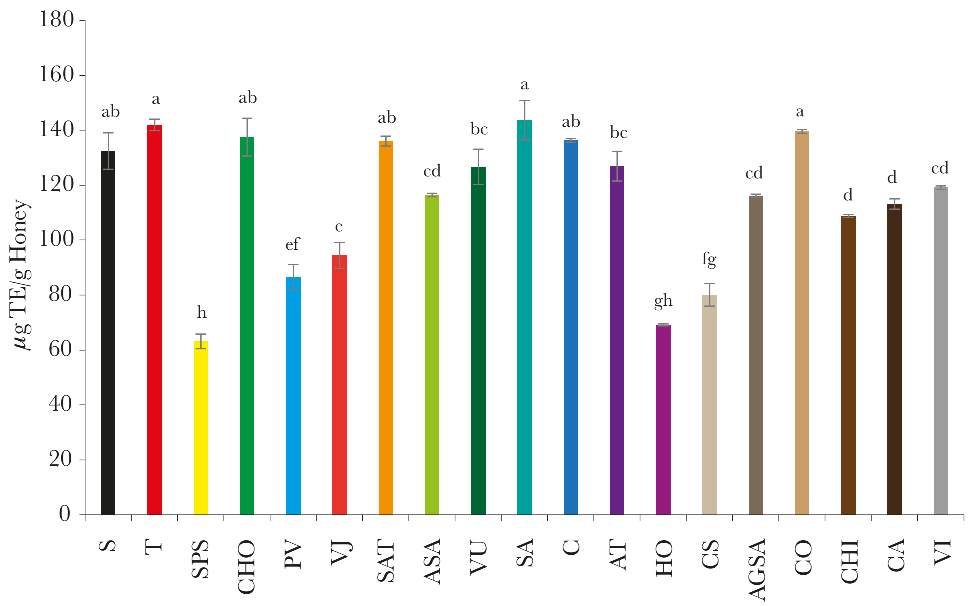

Figure 3. Determination of antioxidant content in honey samples collected in the different regions of the state of Veracruz. Values are presented as mean ± standard deviation; furthermore, different letters in the columns indicate statistically significant differences. Values are presented as mean ± standard deviation; furthermore, different letters in the columns indicate statistically significant differences (one-way ANOVA, $p<0.05$). (S) Soconusco, (T) Texistepec, (SPS), San Pedro Soteapan, (CHO), Cuatotolapan, Hueyapan de Ocampo, (PV), Playa Vicente, (VJ) Villa Juanita, (SAT) San Andrés Tuxtla, (ASA) Almagres, (VU) Valle de Uxpanapa, (SA) Sayula de Alemán, (C), Comejen, (AT), Álamo Temapache, (HO) Hueyapan de Ocampo, (CS) Colmena, Soconusco, (AGSA) Aguilera, (CO) Coatepec, (CHI) Chinameca, (CA) Carlota®, (VI) Vital Real®.

pigments (flavonoids, carotenoids, and polyphenols), enzymes (coenzymes Q, catalases, and oxidases), in addition to nitrogenous compounds, among others (Cianciosi *et al.*, 2018).

The antioxidant content of honey samples from Veracruz presented values between 52-145 μg TE/g honey. The samples that presented the highest contents were from Sayula de Aleman, Texistepec and Coatepec with values between 140,142 and 143 μg TE/g honey, respectively. While the honey samples with the lowest values were from San Pedro Soteapan and Hueyapan de Ocampo, with only 53 and 69 μg TE/g honey, respectively.

It has been reported that clover, eucalyptus and alfalfa honeys present values similar to those shown in the Veracruz honeys analyzed (Cianciosi *et al.*, 2018). Furthermore, as has been reported by various researchers, these antioxidant properties have been linked to both color and moisture content, possibly due to the presence of carotenes and flavonoids (pigments) found in the pollen of the flora where bees percolate (Contreras-Martínez *et al.*, 2020; Chuah *et al.*, 2023).

CONCLUSIONS

In general, the Veracruz honeys had good quality according to the results obtained from the physicochemical analyses, and they are also within the limits of both the national and international regulations that regulate honey. This group of honeys presents a wide spectrum of colors ranging from dark, amber, light amber, extra light to white and aqua white. The color of honey is very important at a commercial level since it defines its price and consequently strongly influences the consumer's purchasing choice. In this sense, the honeys that displayed dark colors were those with the highest contents of total phenols and antioxidants. This suggests that these compounds are directly responsible for providing greater antioxidant capacity and being involved in the color of the honey samples analyzed.

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REFERENCES

Codex Alimentarius Commission—Procedural Manual twenty-seventh edition. 2019. 254. https://doi.org/10.4060/cc5042en

Lichtenberg-Kraag, B. (2014). Evidence for correlation between invertase activity and sucrose content during the ripening process of honey. *Journal of Apicultural Research, 53*(3), 364-373. https://doi.org/10.3896/IBRA.1.53.3.03

Nicolson, S. W., Human, H., & Pirk, C. W. (2022). Honey bees save energy in honey processing by dehydrating nectar before returning to the nest. *Scientific Reports, 12*(1). 16224. DOI:10.1038/s41598-022-20626-5.

Alaerjani, W. M. A., Abu-Melha, S., Alshareef, R. M. H., Al-Farhan, B. S., Ghramh, H. A., Al-Shehri, B. M. A., Bajaber, M. A, Khan, K. A., Alrooqi, M. M., Modawe, G. A., & Mohammed, M. E. A., (2022). Biochemical reactions and their biological contributions in Honey. *Molecules, 27*(15), 4719. https://doi.org/10.3390/molecules27154719

Samarghandian, S., Farkhondeh, T., & Samini, F. (2017). Honey and health: A review of recent clinical research. *Pharmacognosy research. 9*(2), 121. doi: 10.4103/0974-8490.204647

Silva, B., Biluca, F. C., Gonzaga, L. V., Fett, R., Dalmarco, E. M., Caon, T., & Costa, A. C. O. (2021). *In vitro* anti-inflammatory properties of honey flavonoids: A review. *Food Research International*. 141, 110086. https://doi.org/10.1016/j.foodres.2020.110086

Brudzynski, K., & Miotto, D. (2011). Honey melanoidins: Analysis of the compositions of the high molecular weight melanoidins exhibiting radical-scavenging activity. *Food Chemistry. 127*(3), 1023-1030. https://doi.org/10.1016/j.foodchem.2011.01.075

Al-Farsi, M., Al-Amri, A., Al-Hadhrami, A., & Al-Belushi, S. (2018). Color, flavonoids, phenolics and antioxidants of Omani honey. *Heliyon. 4*(10). https://doi.org/10.1016/j.heliyon.2018.e00874

Servicio de Información Agroalimentaria y Pesquera (SIAP). (2022). Cierre de la producción agrícola por estado. Recuperado de https://nube.siap.gob.mx/cierreagricola/

Mexicana N. (2018). NOM-004-SAG/GAN-2018, Producción de miel y especificaciones.

Magaña Magaña, M. A., Tavera Cortés, M. E., Salazar Barrientos, L. L., & Sanginés García, J. R. (2016). Productividad de la apicultura en México y su impacto sobre la rentabilidad. *Revista Mexicana de Ciencias Agrícolas. 7*(5), 1103-1115. https://www.redalyc.org/articulo.oa?id=263146723011

Instituto Nacional de Estadística y Geografía (INEGI). 2020. Climatología. (Climas 1902-2011). México: INEGI. https://www.inegi.org.mx/temas/climatologia/

Elvia J. C., Monserrat D., Leticia G. C. Yolanda S. M., Celestino S. B., & Car-olina G. S. (2015). Anthocyanin and phenolic characterization, chemical composition and antioxidant activity of chagalapoli (*Ardisia compressa* K.) fruit: A tropical source of natural pigments. *Food Research International*. 70, 151-157. https://doi.org/10.1016/j.foodres.2015.01.033

Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). [14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. In Methods in enzymology. 299, 152-178. Academic press. https://doi.org/10.1016/S0076-6879(99)99017-1

Re R., Pellegrini N., Proteggente A., Pannala A., Yang M., & Rice-Evans C. (1999). "Antioxidant activity applying an improved ABTS•+ radical cation decolorization assay". *Free radical biology and medicine. 26* (9-10), 1231-1237. https://doi.org/10.1016/S0891-5849(98)00315-3

Pauliuc, D., & Oroian, M. (2020). Organic acids and physico-chemical parameters of romanian sunflower honey. *Food and Environment Safety Journal. 19*(2). http://fens.usv.ro/index.php/FENS/article/view/720

García-Chaviano, M. E., Armenteros-Rodríguez, E., Escobar-Álvarez, M. D. C., García-Chaviano, J. A., Méndez-Martínez, J., & Ramos-Castro, G. (2022). Composición química de la miel de abeja y su relación con los beneficios a la salud. *Revista Médica Electrónica. 44*(1), 155-167. http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S1684-18242022000100155&lng=es&tlng=en.

Codex Alimentarius Commission—Procedural Manual twenty-seventh edition. (1981). Norma para la miel. CODEX STAN. 12-1981.

Cervera, S. S., & Cervera, M. M. S. (1994). Valores de acidez (Libre, Lactó-nica y Total) y pH de las mieles de la Rioja. *Zubía*. 12, 193-204.

Castillo- Martínez T, García-Osorio C, García-Muñiz JG, Aguilar-Ávila J, & Ramírez-Valverde R. (2022). Sugars and °Brix in honey from *Apis mellifera*, *Melipona beecheii*, and commercial honey from a local market in Mexico. *Veterinaria México OA*. (9): 1-12. https://doi.org/10.22201/fmvz.24486760e.2022.950

Makhloufi, C., Kerkvliet, J. D., D'albore, G. R., Choukri, A., & Samar, R. (2010). Characterization of Algerian honeys by palynological and physi-cochemical methods. *Apidologie. 41*(5), 509-521. https://doi.org/10.1051/apido/2010002

Muñoz Jáuregui, A.M., Alvarado-Ortíz Ureta, C., Blanco Blasco, T., Castañeda Castañeda, B., Ruiz Quiroz, J., & Alvarado Yarasca, Á. (2014). Determinación de compuestos fenólicos, flavonoides totales y capacidad antioxidante en mieles peruanas de diferentes fuentes florales. *Revista de la Sociedad Química del Perú, 80*(4), 287-297. DOI:10.37761/rsqp.v80i4.182

Ávila, S. Silveira, P.,Gerson, L.T., Lovemore, N. M., Apea, F.B., Regina, B, M. Trust, B., & Hoffmann, R. (2019). Bioactive compounds and biological properties of Brazilian stingless bee honey have a strong relationship with the pollen floral origin. *Food Research International.* 123:1-10. https://doi.org/10.1016/j.foodres.2019.01.068

Becerril-Sánchez, A. L., Quintero-Salazar, B., Dublán-García, O., & Escalona-Buendía, H. B. (2021). Phenolic compounds in honey and their relationship with antioxidant activity, botanical origin, and color. *Antioxidants. 10*(11), 1700. https://doi.org/10.3390/antiox10111700

Solayman, M., Islam, M. A., Paul, S., Ali, Y., Khalil, M. I., Alam, N., & Gan, S. H. (2016). Physicochemical properties, minerals, trace elements, and heavy metals in honey of different origins: a comprehensive review. *Comprehensive Reviews in Food Science and Food Safety. 15*(1), 219-233. https://doi.org/10.1111/1541-4337.12182

Hungerford, N. L., Tinggi, U., Tan, B. L., Farrell, M., & Fletcher, M. T. (2020). Mineral and trace element analysis of Australian/Queensland *Apis mellifera* honey. *International journal of environmental research and public health. 17*(17), 6304. https://doi.org/10.3390/ijerph17176304

Aljadi, A. M., & Kamaruddin, M. Y. (2004). Evaluation of the phenolic contents and antioxidant capacities of two Malaysian floral honeys. *Food Chemistry. 85*(4), 513–518. https://doi.org/10.1016/S0308-8146(02)00596-4

Rodríguez, B. A., Mendoza, S., Iturriga, M. H., & Castano-Tostado, E. (2012). Quality parameters and antioxidant and antibacterial properties of some Mexican honeys. *J Food Sci. 77*(1), C121- 127. https://doi.org/10.1111/j.1750-3841.2011.02487.x

Ciappini, M. C., Gatti, M. B., & Di Vito, M. V. (2013). El Color como indicador del contenido de flavonoides en miel. *Revista de Ciencia y Tecnología*. (19), 59-63.http://www.scielo.org.ar/scielo.php?pid=S1851-75872013000100009&script=sci_arttext

Cianciosi, D., Forbes-Hernández, T. Y., Afrin, S., Gasparrini, M., Reboredo-Rodriguez, P., Manna, P. P., Zhang, J., Bravo, L., L., Martínez, F., S., Agudo, T., P., Quiles, J., L., Giampieri, F., & Battino, M. (2018). Phenolic compounds in honey and their associated health benefits: A review. *Molecules. 23*(9), 2322. https://doi.org/10.3390/molecules23092322

Contreras-Martínez, C. S., Macías-Nieves, J. P., García-González, J. M., Trejo-Guardado, V. I., & Carranza-Concha, J. (2020). Antioxidant capacity and phenolic content of bee honey produced in Zacatecas, Mexico. *Revista Fitotecnia Mexicana. 43*(4), 453-453. https://doi.org/10.35196/rfm.2020.4.453

Chuah, W. C., Lee, H. H., Ng, D. H., Ho, A. L., Sulaiman, M. R., & Chye, F. Y. (2023). Antioxidants Discovery for Differentiation of Monofloral Stingless Bee Honeys Using Ambient Mass Spectrometry and Metabolomics Approaches. *Foods. 12*(12), 2404. https://doi.org/10.3390/foods12122404

Colegio de Postgraduados

Distribution of minerals in the organs of green pea and snap bean plants that could be used in Industry 4.0

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ABSTRACT

Objective: To determine the minerals, essential trace elements, toxic trace elements, and rare earth element composition of the organs of green pea (*Pisum sativum*) and snap bean (*Phaseolus vulgaris*) plants that could be potentially used in Industry 4.0.

Design/Methodology/Approach: The concentration of mineral elements was determined through inductively coupled plasma mass spectrometry (ICP-MS). The distribution of minerals in the flours of the different organs (root, stem, leaves, and fruits) of pea (*P. sativum* L.) and snap bean (*P. vulgaris*) was likewise determined.

Results: The leaves are an important fraction of the dry matter (30-40%) of the evaluated plants and they are rich in minerals (calcium, magnesium, phosphorus, and potassium), essential trace elements (manganese, iron, selenium, and zinc), toxic trace elements (aluminum, strontium, boron, tin, and barium), and rare earth elements (cerium, yttrium, lanthanum, and neodymium).

Study Limitations/Implications: The production condition of the crops —on which the mineral elements content largely depend on— is unknown.

Findings/Conclusions: The organs of the pea and snap bean plants contain a significant concentration of minerals, essential trace elements, toxic trace elements, and rare earth elements; therefore, these organs could be used as raw materials for various processes in Industry 4.0.

Keywords: minerals, *Pisum sativum* L., *Phaseolus vulgaris* L.

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INTRODUCTION

By 2050, human population is expected to reach 9.7 billion worldwide [1]. This constant demand for resources will impact the biodiversity of the planet, as well as the health and well-being of the population [2]. Therefore, new sources of affordable and low-cost quality food and biomaterials are required. Additionally new technologies for the management, reduction, and elimination of waste should be introduced and the quality and quantity of food and non-food products should be improved [3].

Mexico has a great diversity of biological resources and their study provides information for a better use of the organs of these plants and for a better management of the agricultural and industrial waste. Although this waste does not represent the main value of the transformation, it can be used as raw material for many other products [4].

Organic waste can help to achieve a nutritional balance based on macro and micronutrients. In order to increase food quality, crops that reduce the vitamin and mineral deficiencies of the population must be included in the diet as a long-term sustainable alternative [5].

The leaves or other organs of plants from the Potosi-Zacatecas highland region are commonly used unaware of their chemical composition. Although they could be unsuitable for human consumption, they could be transformed into medicinal products, cosmetics, and industrial biomaterials [6]. In this sense, their mineral content should be established. In addition, whether or not these minerals are found in harmful concentrations should be determined [3]; these minerals include the so-called rare earths, which have been found in trees, grass, cabbage, and other plants [7].

Currently, rare earth elements are used in banknotes (to prevent counterfeiting), smartphones, green technologies, hybrid cars, wind turbines, military equipment (such as night vision goggles, missiles, and other weapons), etc.

Mineral content depends on various factors, including the species, genetic origin, and geographical location, as well as the organ and the stage of development of the plant. However, the data on the content of mineral elements in different plants are scarce.

The objective of this research was to determine the composition of minerals, essential trace elements, toxic trace elements, and rare earth elements of the organs of green pea (*Pisum sativum*) and snap bean (*Phaseolus vulgaris*) plants that could be potentially used in Industry 4.0.

MATERIALS AND METHODS

Plant material

Nine green pea plants (*Pisum sativum* L.) and nine snap bean plants (*Phaseolus vulgaris* L.) cv 'Pinto Saltillo' were randomly selected for this experiment. The plants were grown in the open air and under rainfed conditions in July 2020. The green pea samples were collected from a plot in Ejido de Moras, Mexquitic de Carmona, San Luis Potosí (22° 29' 62.95" N, −100° 99' 97.97" W). The snap bean plants were collected in a rainfed plot in the municipality of Zacatón, Salinas de Hidalgo, San Luis Potosí (22° 45' 15.3" N, −101° 59' 47.4" W).

Experimental site

The analyses were carried out in the Water-Soil-Plant Laboratory of the Colegio de Postgraduados - San Luis Potosí Campus (22° 63' 22" N and 101° 71' 25" W) and in the Chemistry and Biochemistry Laboratory of the Coordinación Académica Región Altiplano Oeste (CARAO) of the Universidad Autónoma de San Luis Potosí (22° 38' 28.5" N and −101° 42' 10.0" W).

Open acid digestion of samples

The plants were dried at 60 °C, grounded, and stored in Ziploc bags at room temperature until the trials. A 0.5 g sample was weighed on an H-5276 analytical scale (OhausAdventurer) and transferred into a 50 mL flat Teflon tube. Subsequently, 25 ng mL^{-1} of iridium and indium were added to each sample as an internal standard to perform the recovery of the method. In addition, 10 mL of ultra-pure concentrated HNO_3 were added to the mixture, which was kept at room temperature for 12 h.

The samples with the HNO_3 were placed on a BZH29 heating plate (NJBZH) and heated until the evaporation point (hot acid digestion), without allowing it to dry to prevent the loss of mercury (Hg). When the tubes had approximately 1 mL of the concentrated sample, 10 mL of concentrated H_2O_2 were added drop by drop, in order to destroy all the organic matter of the sample (this process is also called total mineralization of the sample). The samples did not require further addition of HNO_3 and/or H_2O_2. Finally, the samples were gauged to 25 mL in class A volumetric flasks with batch certificate.

Determination of mineral elements in ICP-MS

The mineral content was determined with the inductively coupled plasma mass spectrometry (ICP-MS) procedure, using the iCAP™ RQ equipment, in KED (Kinetic Energy Discrimination) mode and with a collision cell [12].

Data analysis

An analysis of variance and a comparison of means (Tukey $\alpha=0.05$) were carried out for each element found in the different organs of the plant. Each species was studied separately, under a completely randomized design. The analysis was carried out in r-project® 4.2.2, using the RStudio® 2023.06.2 interface, both of free distribution.

RESULTS AND DISCUSSION

Distribution of dry matter in the organs of plants

According to their weight, the leaves (395.5 g) of the Pinto-Saltillo bean plant account for 39.55% of the total weight (1 kg). Meanwhile, the weight of the leaves of the pea plant accounts for 30.46% of the total weight of the plant. in both cases this distribution is comparable to the proportion of the weight of the fruits: 33.67% (bean) and 38.44% (plant) (Table 1).

Macromineral composition of the different organs of the snap bean and green pea plants

According to the results, the leaves of pea plants have an outstandingly high concentration of calcium (6.59 mg g^{-1}), magnesium (4.81 mg g^{-1}), and potassium (64.70 mg g^{-1}); the highest concentration of sodium (11.61 mg g^{-1}) and phosphorus (7.52 mg g^{-1}) were found in the root and the fruit, respectively (Table 2). Meanwhile, the leaves of the Pinto-Saltillo bean plant had outstandingly high concentrations of calcium (3.16 mg g^{-1}), magnesium (2.39 mg g^{-1}), and phosphorus (4.66 mg g^{-1}); the highest concentration of sodium (0.15 mg g^{-1}) and potassium (17.09 mg g^{-1}) was recorded in the root and the stem, respectively.

Table 1. Distribution of dry matter in the different organs of snap bean (*Phaseolus vulgaris*) and green pea (*Pisum sativum*) plants.

Specie	Organ	(%)	g kg^{-1}
Phaseolus vulgaris	Root	9.12	91.20
	Stem	17.66	176.60
	Leaves	39.55	395.50
	Fruit	33.67	336.70
Pisum sativum	Root	8.68	86.80
	Stem	22.42	224.20
	Leaves	30.46	304.60
	Fruit	38.44	384.40

Source: Table developed by the authors.

Table 2. Macromineral concentration in different organs of snap bean (*P. vulgaris*) and green pea (*P. sativum*) plants.

Specie	Organ	Mineral content (mg g^{-1})				
		Calcium (Ca)	Magnesium (Mg)	Potassium (K)	Sodium (Na)	Phosphorus (P)
Phaseolus vulgaris	Root	2.16±0.212	0.77±0.001	9.93±0.234	0.15±0.001	2.95±0.056
	Stem	1.15±0.003	1.00±0.001	17.09±0.001	0.09±0.001	4.26±0.001
	Leaves	3.16±0.003	2.39±0.012	11.23±0.001	0.13±0.001	4.66±0.001
	Fruit	0.23±0.003	1.42±0.074	10.62±0.001	0.06±0.001	4.53±0.001
Pisum sativum	Root	2.01±0.003	1.82±0.002	38.02±0.001	11.61±0.010	5.18±0.001
	Stem	5.67±0.003	4.14±0.002	53.10±0.001	5.07±0.005	4.17±0.001
	Leaves	6.59±0.003	4.81±0.003	64.70±0.001	5.95±0.029	4.82±0.001
	Fruit	0.35±0.003	1.52±0.001	10.07±0.001	0.11±0.001	7.52±0.001

Source: Table developed by the authors. In all cases, a significant difference was found ($p<0.001$), but no clustering was recorded (Tukey, 0.05).

The leaves and fruits of *P. vulgaris* recorded the following concentrations: 10.9 to 16.4 mg g^{-1} of calcium, 2.4 to 3.3 mg g^{-1} of magnesium, and 3.1 to 3.8 mg g^{-1} of potassium. Meanwhile, the snap bean pod recorded values of 0.00018 mg g^{-1}, 0.00142 mg g^{-1}, 0.00033 mg g^{-1}, and 0.00053 mg g^{-1} of phosphorus, potassium, magnesium, and calcium, respectively [14]. These results are different from those found in this research for the fruit of *P. vulgaris*: 4.53 mg g^{-1} of phosphorus, 1.42 mg g^{-1} of magnesium, and 0.23 mg g^{-1} of calcium.

Other researchers [15] determined that pea (*P. sativum*) pods had magnesium values of 2.10 mg g^{-1}. Although this research obtained similar values (1.52 mg g^{-1}), calcium values (7.70 mg g^{-1}) were different (0.35 mg g^{-1}). Likewise, other authors reported 1.03 mg g^{-1} of magnesium, 11.35 mg g^{-1} of calcium, and 10.44 mg g^{-1} of potassium in green peas [16].

Meanwhile, 1.62 mg g^{-1} magnesium concentrations were recorded in pea seeds from various populations, these results are similar to the values recorded in this research (1.52 mg g^{-1}). Meanwhile, Hacisalihoglu, Beiselm, and Settles (2021) [12] reported similar magnesium (0.90 to 1.40 mg g^{-1}) and calcium (0.56 to 0.90 mg g^{-1}) values in pea seeds than those recorded in this research (0.34 mg g^{-1} of calcium and 1.52 mg g^{-1} of magnesium. The difference between the values of this research and the findings of previous research works may be due to several factors, including: variety, techniques, equipment and solvents used to determine the content of these minerals, climatic and cultivation conditions, and postharvest storage.

Composition of essential trace elements of the different organs of the snap bean and green pea plants

Snap bean leaves had a higher content of iron (437.14 mg g^{-1}), manganese (237.77 mg g^{-1}), copper (14.04 mg g^{-1}), and selenium (160.45 ng g^{-1}) than pea leaves. Meanwhile the root of the pea plant recorded the highest concentration of chromium (25.35 mg g^{-1}) and cobalt (0.30 mg g^{-1}). Finally, the stem presented the highest concentration of zinc (20.03 mg g^{-1}).

Pea leaves had a higher content of manganese (275.84 mg g^{-1}), iron (653.51 mg g^{-1}), copper (18.25 mg g^{-1}), zinc (74.49 mg g^{-1}), and selenium (312.42 ng g^{-1}) than snap bean leaves. Meanwhile the fruit of the green pea plant had the highest concentration of chromium (48.20 mg g^{-1}) and cobalt (0.59 mg g^{-1}) (Table 3).

On the one hand, the element with the highest concentration in the 8 samples evaluated was iron, particularly in the stem of the green pea plant (664.74 mg g^{-1}), followed by manganese, especially in the leaves of the snap bean plant (275.84 mg g^{-1}).

On the other hand, the values of the snap bean plants are similar to the snap bean pod: 5.61 mg g^{-1} of copper, 25.21 mg g^{-1} of manganese, 73.45 mg g^{-1} of iron, and 19.28 mg g^{-1} of zinc [14]. Likewise, the following concentrations were found in *P. vulgaris* fruits: 6,004 to 1,474 mg g^{-1} of iron , 312 to 557 mg g^{-1} of manganese, 30.4 to 43.7 mg g^{-1} of zinc, 5.7 to 30.5 mg g^{-1} of copper, 2.7 to 4.69 mg g^{-1} of chromium, 1.9 to 3.1 mg g^{-1} of nickel, and 915 to 2,152 mg g^{-1} of aluminum [13]. The following concentrations were found in various green pea seeds: iron (67.49 mg g^{-1}), zinc (49.70 mg g^{-1}), and copper (6.60 mg g^{-1}) [12]. These results are also similar to the findings of this research: iron (91.58

Table 3. Concentration of essential trace elements in different organs of snap bean (*P. vulgaris*) and green pea (*P. sativum*) plants.

Specie	Organ	Trace elements (mg g^{-1})						
		Cr	Mn	Fe	Co	Cu	Zn	*Se (ng g^{-1})
P. vulgaris	Root	25.35±0.64^a	39.96±0.08^c	169.95±0.96^b	0.30±0.01^a	4.40±0.39^b	14.86±0.89^c	33.67±0.64^b
	Stem	8.73±0.02^c	37.20±1.16^c	91.58±0.04^c	0.16±0.01^b	4.16±0.07^b	16.21±0.01^b	16.59±0.18^b
	Leaves	19.90±0.13^b	237.77±0.01^a	437.14±0.10^a	0.29±0.05^a	14.04±0.04^a	14.95±0.02^c	160.45±0.32^a
	Fruit	4.99±0.03^d	47.71±0.22^b	64.60±0.16^d	0.08±0.01^c	4.62±0.02^b	20.03±0.02^a	19.21±0.14^c
P. sativum	Root	0.73±0.01^d	72.75±0.18^c	304.33±0.03^b	0.30±0.04^d	14.75±0.06^c	33.89±0.03^c	200.85±1.29^c
	Stem	48.20±0.17^a	63.23±0.02^d	157.53±0.04^c	0.59±0.01^a	6.93±0.02^d	26.80±0.24^d	21.34±0.63^d
	Leaves	1.81±0.01^c	275.84±0.08^a	653.51±0.18^a	0.34±0.08^c	18.25±0.07^a	74.49±0.24^a	312.42±2.70^a
	Fruit	9.84±0.03^b	242.72±0.23^b	664.74±45.87^a	0.38±0.01^b	17.77±0.16^b	46.33±0.21^b	237.69±2.37^b

*Expressed in nanograms per gram. Source: Table developed by the authors. In all cases a significant difference was found ($p<0.001$). Means with the same letter in each column for each species do not record a significant difference (Tukey, 0.05).

mg g^{-1}), zinc (16.21 mg g^{-1}), and copper (4.16 mg g^{-1}). The values reported in another research work [16] for zinc (38.80 mg g^{-1}) and iron (33.10 mg g^{-1}) in green peas are similar to the results of this research for both minerals (49.70 mg g^{-1} of zinc and 67.49 mg g^{-1} of iron). Other publications [17] have reported similar values for copper (7.00 mg g^{-1}), iron (70.00 mg g^{-1}), and zinc (30.03 mg g^{-1}) in green pea seeds than those reported in this research for copper (4.16 mg g^{-1}), iron (91.58 mg g^{-1}), and zinc (16.21 mg g^{-1}). Likewise, other researchers [18] report the following concentrations in green pea seeds: copper (10.7 mg g^{-1}), zinc (39.6 mg g^{-1}), iron (53.8 mg g^{-1}), and manganese (16.6 mg g^{-1}). Those results are also similar to the findings of this research for copper (4.16 mg g^{-1}), zinc (16.21 mg g^{-1}), iron (91.58 mg g^{-1}), and manganese (37.20 mg g^{-1}).

Composition of toxic trace elements of the different organs of the snap bean and green pea plants

On the one hand, according to the results, the leaves of the snap bean plant are the product with the highest amount of toxic trace elements, particularly aluminum (708.52 mg g^{-1}), titanium (93.08 mg g^{-1}), barium (15.53 mg g^{-1}), and boron (10.40 mg g^{-1}). Meanwhile, the root has high concentrations of strontium (122.70 mg g^{-1}), nickel (14.44 mg g^{-1}), and lithium (7.20 mg g^{-1}). The fruit has high concentrations of tin (56.10 mg g^{-1}) (Table 4).

On the other hand, green pea leaves have a higher concentration of aluminum (806.33 mg g^{-1}), strontium (263.47 mg g^{-1}), boron (69.00 mg g^{-1}), barium (43.52 mg g^{-1}), and lithium (5.39 mg g^{-1}). Meanwhile, the fruit has a higher concentration of tin (44.87 mg g^{-1}), nickel (32.18 mg g^{-1}), and titanium (8.80 mg g^{-1}).

The toxic trace elements with the highest concentration in the two varieties were aluminum, strontium, boron, tin, titanium, nickel, and barium. The toxic trace elements with $<$10 mg g^{-1} concentrations were lithium, gallium, lead, bismuth, arsenic, antimony, vanadium, cadmium, zirconium, niobium, silver, tantalum, tungsten, mercury, thorium, and uranium.

Table 4. Concentration of toxic trace elements in different organs of snap bean (*P. vulgaris*) and green pea (*P. sativum*) plants.

Specie	Organ	Mineral content (mg g^{-1})							
		Lithium (Li)	Boron (B)	Aluminum (Al)	Nickel (Ni)	Strontium (Sr)	Tin (Sn)	Barium (Ba)	titanium (Ti)
Phaseolus vulgaris	Root	7.20±0.20^{a}	2.58±0.21^{d}	111.19±0.20^{b}	14.44±0.60^{a}	122.70±0.30^{a}	37.49±1.47^{b}	4.32±0.10^{c}	51.39±0.98^{b}
	Stem	0.10±0.01^{c}	5.61±0.02^{b}	35.06±0.29^{d}	6.83±0.04^{c}	14.56±0.18^{d}	56.10±0.02^{a}	2.86±0.01^{d}	5.89±0.09^{d}
	Leaves	0.86±0.01^{b}	10.40±0.01^{a}	708.52±0.29^{a}	11.19±0.08^{b}	118.06±0.01^{b}	36.54±0.04^{b}	15.53±0.03^{a}	93.08±0.30^{a}
	Fruit	0.87±0.01^{b}	4.15±0.03^{c}	72.49±0.29^{c}	3.49±0.04^{d}	67.12±0.02^{c}	28.17±0.07^{c}	7.04±0.02^{b}	27.48±0.25^{c}
Pisum sativum	Root	2.87±0.01^{c}	28.40±0.02^{c}	453.91±0.29^{c}	0.74±0.01^{d}	115.92±0.02^{c}	---------	16.99±0.03^{b}	---------
	Stem	0.44±0.01^{d}	3.98±0.01^{d}	48.18±0.29^{d}	32.18±0.01^{a}	19.85±0.03^{d}	44.87±0.05	2.43±0.01^{d}	8.80±0.35
	Leaves	5.39±0.01^{a}	69.00±0.01^{a}	806.33±0.29^{a}	1.60±0.01^{c}	263.47±0.01^{a}	---------	43.52±0.1^{c}	---------
	Fruit	4.35±0.02^{b}	61.18±0.01^{b}	657.73±0.29^{b}	1.92±0.02^{b}	216.44±124.9^{b}	---------	33.82±0.09^{a}	---------

Source: Table developed by the authors. In all cases a significant difference was found ($p<0.001$). Means with the same letter in each column for each species do not have a significant difference (Tukey, 0.05).

These results contribute to a more complete and accurate information about the mineral content. An optimal use of the different organs of the green pea and snap bean plants is achieved in the different chemical, biological, and technical processes.

There is little information about the toxic trace elements in the different organs of the green pea and snap bean plants. However, other researchers [14] have reported similar nickel values (4.00 mg g^{-1}) in snap bean pods to this research (6.83 mg g^{-1}). Meanwhile, different boron concentrations (9.69 mg g^{-1}) in various green pea seeds were found in other research works; these results differ from the findings of this research regarding the fruit (3.98 mg g^{-1}).

Composition of the rare earth elements in the different organs of snap bean and green pea plants

The snap bean leaves recorded significant concentrations of cerium (947.94 ng g^{-1}), yttrium (808.05 ng g^{-1}), neodymium (440.04 ng g^{-1}), and lanthanum (437.6 ng g^{-1}). The other elements are found in concentrations of $<$100 ng g^{-1}.

Meanwhile, the only organ of the green pea plant that has a concentration of rare earth elements is the fruit (pod). As in the previous case, cerium recorded the highest concentration (71.86 ng g^{-1}), followed by yttrium (48.63 ng g^{-1}), lanthanum (30.30 ng g^{-1}), and neodymium (28.52 ng g^{-1}). The other elements are found in concentrations of $<$7 ng g^{-1} (Table 5).

Table 5. Concentration of rare earth elements in different organs of snap bean (*P. vulgaris*) and green pea (*P. sativum*) plants.

Specie	***Phaseolus vulgaris***				***Pisum sativum***
Rare-earth element (ng g^{-1})	**Root**	**Stem**	**Leaves**	**Fruit**	**Fruit**
Yttrium (Y)	151.45±1.44	72.07±0.77	808.05±2.74	36.18±0.24	48.63±0.79
Lanthanum (La)	84.01±1.31	41.74±0.30	437.60±9.47	25.53±0.39	30.30±0.18
Cerim (Ce)	207.49±2.67	102.78±1.08	947.94±10.72	68.48±0.25	71.86±0.21
Praseodymium (Pr)	19.62±0.57	9.27±0.06	117.34±0.06	6.04±0.06	6.48±0.17
Neodymium (Nd)	79.17±2.23	38.48±0.40	440.31±0.2	27.51±0.16	28.52±0.45
Samarium (Sm)	17.27±1.77	7.45±0.20	96.33±0.21	5.38±0.16	5.26±0.16
Europium (Eu)	0.17±0.03	---	4.62±0.35	---	---
Gadolinium (Gd)	16.58±0.63	7.27±0.22	92.56±0.18	5.05±0.05	5.12±0.15
Terbium (Tb)	0.90±0.13	---	12.26±0.12	---	---
Dysprosium (Dy)	14.87±2.34	6.26±0.04	77.45±0.08	4.75±0.20	4.62±0.27
Holmium (Ho)	1.28±0.28	---	13.76±0.23	---	---
Erbium (Er)	8.36±0.58	3.54±0.13	42.62±0.33	2.68±0.10	2.72±0.19
Thulium (Tm)	---	---	4.33±0.29	---	---
Ytterbium (Yb)	5.91±0.94	1.65±0.11	36.68±0.38	1.12±0.18	0.80±0.19
Lutetium (Lu)	0.06±0.03	---	4.20±0.28	---	---

Source: Table developed by the authors.

CONCLUSIONS

The weight ratio of the leaves of the green pea and snap bean plants is comparable to the weight of the fruit. Additionally, these leaves have the highest concentration of such minerals as calcium, magnesium, phosphorus, and potassium, as well as essential trace elements, including manganese, iron, selenium, and zinc. The toxic trace elements with the highest concentration in the leaves of both species are aluminum, strontium, boron, tin, and barium. Meanwhile the highest concentration of rare earth elements (cerium, yttrium, lanthanum, and neodymium) was found in the snap bean leaves and the green pea fruit.

The organs of green pea and snap bean plants are an alternative raw material for the food and biomaterial production enriched with minerals, essential trace elements, toxic trace elements, and rare earth elements, which accumulate and bioconcentrate in these organs. In conclusion, these plants can be used as indicators or as phytoextractors which can be selected as inputs in Industry 4.0 processes.

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REFERENCES

1. ONU, 2022. Organización de las naciones unidas. Población mundial | Fondo de Población de las Naciones Unidas. Recuperado 13 de mayo de 2022 (https://www.unfpa.org/es/data/world-population-dashboard).
2. Diamond, J. 2006. Colapso. Por qué unas sociedades perduran y otras desaparecen. Barcelona, Debate. ISBN: 84-8306-648-3. 747 p.
3. Sheldon, R. A. (2000). Atom efficiency and catalysis in organic synthesis. *Pure and Applied Chemistry, 72*(7):1233-1246. https://doi.org/10.1351/pac200072071233
4. Saval, S. (2012). Aprovechamiento de residuos agroindustriales: pasado, presente y futuro. *Revista de la Sociedad Mexicana de Biotecnología y Bioingeniería A. C, 16*(2), 14-46. https://www.academia.edu/23775116/Aprovechamiento_de_Residuos_Agroindustriales_Pasado_Presente_y_Futuro
5. Pachón, H.2010. El impacto nutricional de cultivos biofortificados o cultivos con mayor calidad nutricional [WorkingPaper]. AgroSalud; Centro Internacional de Agricultura Tropical (CIAT). https://cgspace.cgiar.org/handle/10568/70022
6. Özcan, M., Ünver, A., Uçar, T., & Arslan, D.2008. Mineral content of some herbs and herbal teas by infusion and decoction. *FoodChemistry*, 106, 1120-1127. https://doi.org/10.1016/j.foodchem.2007.07.042
7. Tyler, G. 2004. Rare earth elements in soil and plant systems – A review. *Plant and Soil. 267*(1):191-206. (https://link.springer.com/article/10.1007/s11104-005-4888-2)
8. Prego Reboredo, R. M. (2019). Las tierras raras: CSIC, Consejo Superior de Investigaciones Científicas, 2019 - 141 p. - ¿Qué sabemos de...?; 99 - ISBN: 9788400104634 - Permalink: http://digital.casalini.it/9788400104634 - Casalini id: 4452076
9. Sánchez-Castillo C.P., Dewey P.J.S., Aguirre A., Lara J.J., Vaca R., León de la Barra P., Ortiz M., Escamilla I. & James, W.P.T. (1998). The mineral content of Mexican fruits and vegetables. *Journal of Food Composition and Analysis.11*(4): 340-56. https://doi.org/10.1006/jfca.1998.0598
10. de Oliveira, MW, Bhatt, R., Lessa, JB, Santos, D. de F., de Assis, WO y Tenório, TM (2023). Eliminación de nutrientes y calidad bromatológica del maíz en cultivos de alta productividad. *DELOS: DESARROLLO LOCAL SOSTENIBLE , 16* (43), 525–543. https://doi.org/10.55905/rdelosv16.n43-004
11. Jakubowska, M., Zembrzuski, W., and Lukaszewski, Z. (2006). Oxidative extraction versus total decomposition of soil in the determination of thallium. *Talanta, 68*(5), 1736-1739. https://doi.org/10.1016/j.talanta.2005.08.035
12. Hacisalihoglu G, Beisel N.S.& Settles A.M. (2021). Characterization of pea seed nutritional value within a diverse population of *Pisum sativum*. *PLoSOne. 16*(11): e0259565. https://doi.org/10.1371/journal.pone.0259565

13. Hernández A. J.; Alexis, S.; Fernández-Pascual, M.; Pastor, J. 2006. Estudio de la nutrición mineral de Phaseolus vulgaris L. en suelos de cultivo que contienen metales pesados. En: Nutrición Mineral. Aspectos fisiológicos, agronómicos y ambientales. C. Lamsfus, (EditoraCoordinadora): 573-580 Ed. Universidad Pública de Navarra, Pamplona. ISBN: 84-9769-165-2. https://digital.csic.es/bitstream/10261/52096/1/Pamplona.pdf
14. Fernández Valenciano, A. F., y Sánchez Chávez, E. (2017). Estudio de las propiedades fisicoquímicas y calidad nutricional en distintas variedades de frijol consumidas en México. *Nova scientia, 9*(18), 133-148. https://doi.org/10.21640/ns.v9i18.763
15. Mateos-Aparicio, I., Redondo-Cuenca, A., Villanueva-Suárez, M. J., Zapata-Revilla, M. A., and Tenorio-Sanz, M. D. (2010). Pea pod, broad bean pod and okara, potential sources of functional compounds. *LWT - Food Science and Technology, 43*(9), 1467-1470. https://doi.org/10.1016/j.lwt.2010.05.008
16. Millar, K. A., Gallagher, E., Burke, R., McCarthy, S., and Barry-Ryan, C. (2019). Proximate composition and anti-nutritional factors of fava-bean (*Vicia faba*), green-pea and yellow-pea (*Pisum sativum*) flour. Subtropical Plant Science. https://10.1016/j.jfca.2019.103233
17. Woźniak, A., Makarski, B., Stepniowska, A. & Soroka, M. (2012). Chemical composition of pea (*Pisum sativum* L.) seeds depending on tillage systems. *Journal of Elemntology*. 19. DOI: 10.5601/jelem.2014.19.3.484
18. Ton, A., Mart, D., Karaköy, T., Türkeri, M., Torun, A. A. & Anlarsal, A. E. (2022) "Characterization of some local pea (*Pisum sativum* L.) genotypes for agro-morphologicaltraits and mineral concentrations," *Turkish Journal of Agriculture and Forestry*: 46: 2, 11. https://doi.org/10.55730/1300-011X.2975

Colegio de Postgraduados

Physicochemical quality of underground water from agricultural influence

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ABSTRACT

Groundwater in rural areas is the only source of supply for consumption and various activities. It is exposed to various pollutants that arrive through the subsoil layers. The objective of this study was to evaluate the quality of water from 15 wells in the municipality of Cotaxtla, through physicochemical variables such as pH, conductivity (EC), salinity and total dissolved solids (TDS), where the results were compared with the regulations according to its use. The samples were collected according to NOM-230-SSA1-2002. The physicochemical variables were determined with the multiparametric Consort C6010. Results ranged from pH 6.71-8.04, EC 228-4500 mS/cm, salinity 0.13-2.42 mg/L, and TDS 132-2250 mg/L. In every case, water is destined for agricultural use, where 52% is used for livestock and 80% for consumption, 60% of the wells are community and supply 612 inhabitants. According to Mexican regulations, the results obtained from EC, 13% of the wells were not suitable for consumption, in relation to the TDS results, 33% were not suitable for consumption and 13% were not suitable for agricultural irrigation. The pH showed values within the norm, however, one showed a pH of 6.71±0.043, while the rest were found between 7.4±0.03-8.1±0.19. In all values, significant differences were shown between the sites analyzed.

Keywords: Infiltration, groundwater table, water use.

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INTRODUCTION

During recent years, the increase in anthropogenic activities has caused deterioration in water quality. Globally, 20% of the population does not have access to drinking water and close to 50% does not have adequate sanitation [1]. However, more than 50% of the population depends on underground water; in rural zones, underground water is the sole source of supply destined to primary activities, domestic use and consumption [2]. However, it is also exposed to incoming contaminant compounds that reach the water table, thus affecting the water quality [3]. The impact that they cause happens through the subsoil layers, where contaminants manage to dissolve substances as they infiltrate, forming undesirable compounds [4]. The main sources of underground water pollution can come primarily from industrial activities, agriculture and livestock production, although the intensive use of fertilizers and agrichemicals in agricultural zones can be a diffuse source of pollution.

Cotaxtla, located in the Sotavento plains, in the central coastal zone of the state of Veracruz, is a municipality devoted primarily to agriculture and its only source of drinking water supply comes mainly from underground water supplied by the Cotaxtla aquifer [5]. The aquifer is reloaded moslty from infiltration of rainwater that falls in the valley and along the main rivers (Jampa River and Cotaxtla River), and induced from infiltration of the agricultural irrigation excess produced along the irrigation canals [6]. Therefore, this study had the objective of evaluating the physicochemical quality of underground water and its relationship with the maximum permissible limits specified in the national and international regulations according to its use.

MATERIALS AND METHODS

The study zone is the municipality of Cotaxtla, located in coordinates 18° 50' latitude North and 96° 24' longitude West (Figure 1). It has a territorial extension of 537.8 km^2, of which 203 km^2 are used for agriculture [7]. A total of 15 sampling points were selected from 9 localities: Paso Faisán (PF), Las Lomitas (LL), Paso Anona (PA), Bajo Tlachiconal (BT), Mundo Nuevo (MN), Loma Angosta (LA), Mata Tejón (MT), Mata Tambor (MTB), and La Aurora (LAU). A survey was carried out with the aim of understanding the characteristics of the wells, such as total depth, time when it was built, type of material with which it was built, and uses to which underground water is destined.

Sampling of underground water was done in the months of September to November 2022. The samples were collected according to the NOM-230-SSA1-2002 [8]; they were obtained after letting the water run for 3 minutes, rinsing the container before collecting the sample. Polyethylene containers with 500 mL capacity were used, which were identified by sampling point and locality, and then were conserved in a refrigerator at 4 °C and

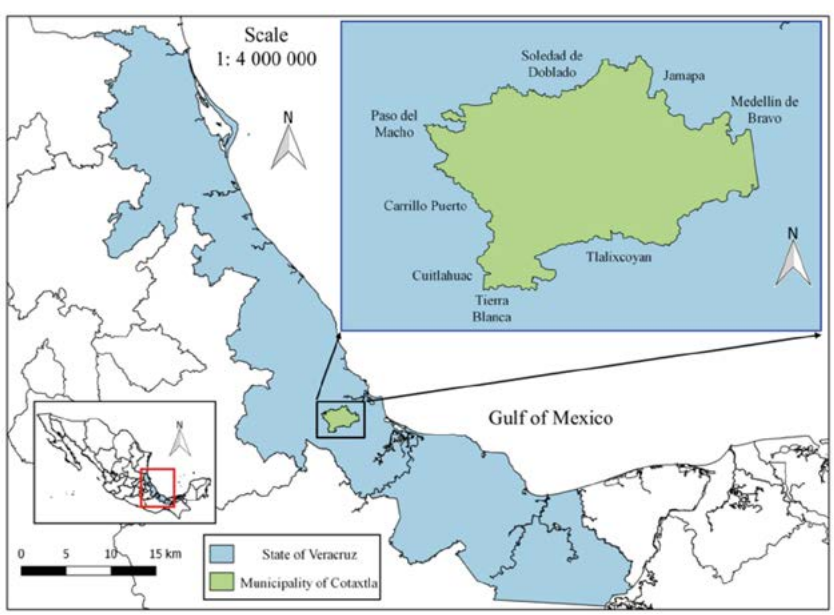

Figure 1. Location of the study area.

transported to the Aquatic Resources Research Laboratory (*Laboratorio de Investigación en Recursos Acuáticos*, LIRA) of Instituto Tecnológico de Boca del Río.

The physicochemical variables analyzed were pH, electric conductivity (EC), total dissolved solids (TDS), and salinity (S), measured using a multiparametric catheter Consort C6010, through three successive independent readings. Descriptive statistics were applied for data analysis through the PAST program, to summarize the data obtained in this study. The minimal values, maximum values and mean ± SD of the physicochemical variables (pH, salinity, EC and TDS) of underground water were obtained. In addition, a normality test was applied, to understand the distribution of the data obtained. The Shapiro-Wilk test and the Kruskal-Wallis test were applied to determine if there are significant differences, for which the PAST program was used [9, 10]. The results were compared with the established permissible limits from the national and international normativity according to its use (Table 1).

RESULTS AND DISCUSSION

The depth of the wells analyzed ranged between 20 and 100 m, where water is extracted through a pumping system. The survey results indicated that 100% of the wells analyzed are destined primarily to agricultural activity, 52% are used for livestock production, and 80% are used for domestic activities and consumption (Figure 2); 60% correspond to community wells that supply 612 inhabitants.

According to the Shapiro-Wilk test, it was seen that the physicochemical data did not show a normal distribution ($p<0.05$); however, significant differences were observed in all the parameters evaluated and regarding all the sampling points according to the Kruskal-Wallis test.

The pH results ranged between 6.71±0.043 and 8.04±0.19, with a mean of 7.68 (Figure 3a). Of the wells, 93% had a pH above neutrality. The results are like those reported

Table 1. Maximum permissible limits for water quality of the national and international normativity according to its use.

Normativity	Use	pH	SDT (mg/L)	CE (mS/cm)
NOM-127-SSA1-2021 [11]	Human consumption	6.5-8.5	1000	-
CE-CCA-001 [12]	Agricultural irrigation	4.5-9	500	-
WHO [13]	Human consumption	6.5-8.5	200	720

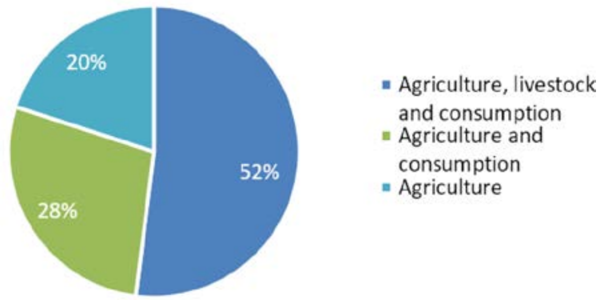

Figure 2. Main uses of underground water in the sampling sites.

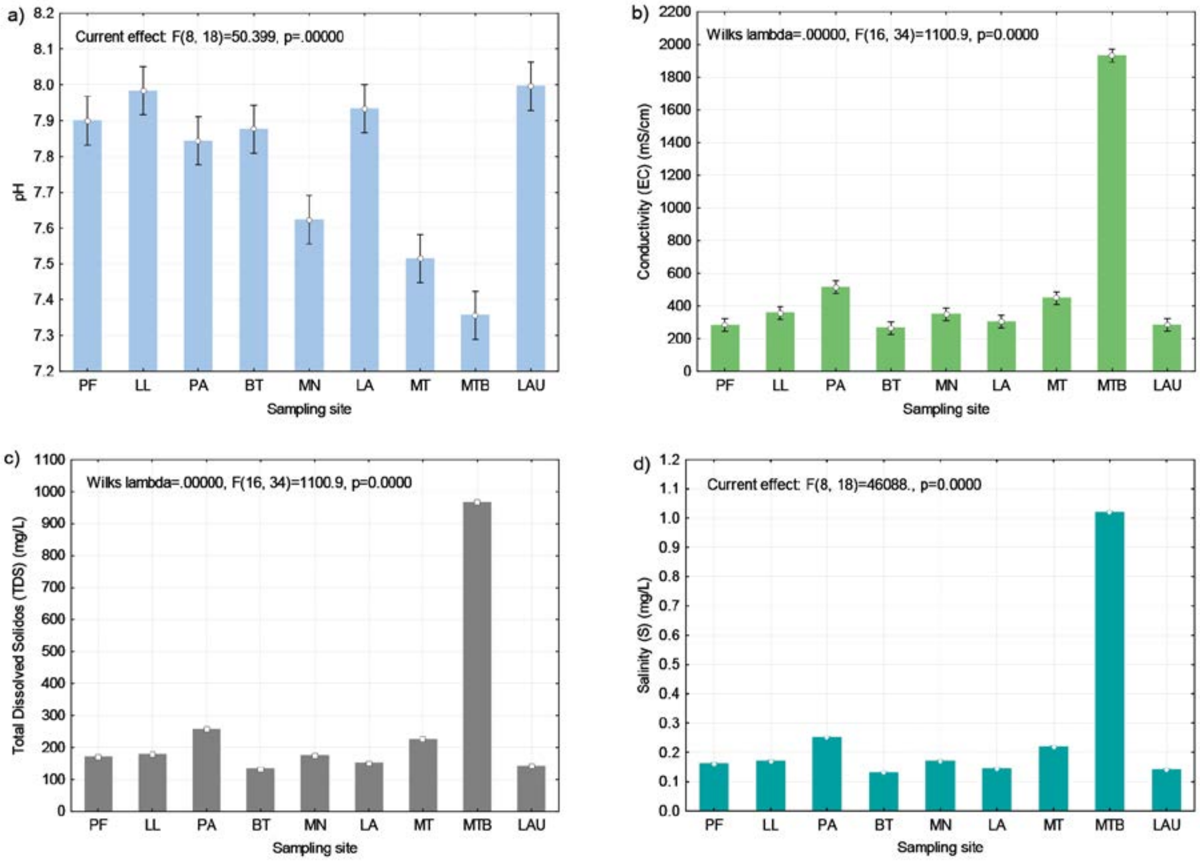

Figure 3. Variation of physicochemical variables in underground water of the municipality of Cotaxtla, Veracruz, Mexico. a) pH, b) conductivity (EC) in mS/cm, d) Total Dissolved Solids (TDS) in mg/L, d) salinity (S) in mg/L.

by Sánchez *et al.* (2016) [14], who found values of 7.15-7.63 in underground water from Quintana Roo, Mexico; it was the same case with Rauf *et al.* (2021) [15] who reported pH values of 6.5-8.8 with a mean of 7.65. However, the pH values of this study were found within the maximum permissible limits for agricultural consumption and irrigation according to those established by the national and international normativity (Table 1).

Regarding the EC results, values between 228.15±2.08 and 4500±5.033 mS/cm with a mean of 681 mS/cm (Figura 3b) were detected; they were lower than those reported by Vijayakumar *et al.* (2022) [16] who found a mean of 1067.65 mS/cm. The EC of the samples depends on the concentrations of various species of ions and their capacity to transport energy in a solution, which is affected by the presence of metal ions in the water [17].

The high concentrations of EC in underground water are due primarily to soluble salts that reach through the infiltration of soil layers [18]. According to WHO (2006) [13], the values should not exceed the 750 μS/cm for the water to be optimal for human consumption, which is why 13% of the wells were not apt for consumption.

According to the water quality guidelines of the consulting committee for agricultural irrigation from the University of California, the water has a mean risk of 750-1500 mS/cm, while a higher risk is 1500-3000 mS/cm [19]. The results of total dissolved solids (TDS) are between 132 and 2247 mg/L, with a mean of 344 mg/L (Figure 3c), where the highest concentrations were observed in the MTB2 and MTB3 wells of the locality of Mata Tambor. The results agree with those reported by Vijayakumar *et al.* (2022) [16], who found values between 212 and 1905 mg/L, with a mean of 751.3 mg/L; likewise, Lanjwani *et al.* (2020) [18] found higher concentrations, of 318 to 7411 mg/L. The presence of TDS is due to the elements, minerals, salts, anions, and cations dissolved in the water sample. The high concentration of total dissolved solids can cause stomach irritation and its prolonged use can cause cardiac disease and kidney stones in humans [18]. However, the normativity by the WHO [13] establishes values with a maximum permissible limit of 200 mg/L for drinking water; therefore, 33% of the samples were not apt for consumption and 13% were not apt for agricultural irrigation. The TDS can also be affected by the geological nature of the soil present in underground water (Lanjwani *et al.*, 2020) [18].

The salinity results observed in this study varied between 0.13 and 2.42 ± 0.005 mg/L, with a mean of 0.35 mg/L (Figure 3d). The salinity in underground water is associated with the intrusion of marine water and various anthropogenic activities; however, in irrigation water it can cause serious problems in certain crops and affect the growth of the plants [15]. The results from this study were similar to those reported by CONAGUA (2020) [6], who analyzed the underground water of the Cotaxtla aquifer, with pH values of 6.76-7.32, EC values of 220-930 mS/cm, and TDS values of 110-460 mg/L; they reported that these values are within the Mexican normativity for human consumption NOM-127-SSA1-2021 [11].

CONCLUSIONS

The variation of the parameters analyzed in this study indicated that underground water from the municipality of Cotaxtla has great influence from the strong agricultural activity in the zone, where variation of these parameters is due to intensive agricultural practices from the use of fertilizers and agrichemicals that are infiltrated through the subsoil layers.

In addition to this, the 80% destined to consumption in the zone, and which supplies more than 612 inhabitants, indicated that according to the normativity for EC values, 13% of the wells were not apt for consumption, while according to the TDS values, 33% were not apt for consumption.

Likewise, the increase in salts present in the underground water can have effects on the crops, and they indicated that 13% were not apt for agricultural irrigation according to the levels of TDS and of EC.

It is recommended to take precautions for the management of underground water consumption, to opt for preliminary water treatments before consumption, as well as to manage the control of the excessive use of organic fertilizers and agrichemicals that alter the chemical quality of the underground water.

REFERENCES

1. Cosgrove, W. J., & Rijsberman, F. R. (2014). World water vision: making water everybody's business. Routledge. https://doi.org/10.4324/9781315071763

2. Nouri, J., Mahvi, A., Jahed, G., & Babaei, A. (2008). Regional distribution pattern of groundwater heavy metals resulting from agricultural activities. *Environmental Geology, 55*(6), 1337-1343. https://doi.org/10.1007/s00254-007-1081-3
3. Mahato, M. K., Singh, G., Singh, P. K., Singh, A. K., & Tiwari, A. K. (2017). Assessment of mine water quality using heavy metal pollution index in a coal mining area of Damodar River Basin, India. *Bulletin of environmental contamination and toxicology, 99*(1), 54-61.
4. Navarro-Aviñó, J. P., Alonso, I. A., & López-Moya, J. (2007). Aspectos bioquímicos y genéticos de la tolerancia y acumulación de metales pesados en plantas. *Ecosistemas, 16*(2). https://www.revistaecosistemas.net/index.php/ecosistemas/article/view/125
5. SEFIPLAN. (2015). Cuadernillos municipales. Retrieved Diciembre from http://www.veracruz.gob.mx/wp-content/uploads/sites/2/2015/05/Cotaxtla.pdf
6. CONAGUA. (2020). Actualización de la disponibilidad media anual de agua en el acuifero cotaxtla (3008), Estado de Veracruz. Gerencia de Aguas Subterráneas. Retrieved Noviembre from https://sigagis.conagua.gob.mx/gas1/Edos_Acuiferos_18/veracruz/DR_3008.pdf
7. INAFED, I. N. p. e. F. y. D. M. (2016). Cotaxtla. Retrieved Diciembre from http://www.inafed.gob.mx/work/enciclopedia/EMM30veracruz/municipios/30049a.html
8. DOF (2002). NOM-230-SSA1-2002. Agua para uso y consumo humano, requisitos sanitarios que se deben cumplir en los sistemas de abastecimiento públicos y privados durante el manejo del agua. Procedimientos sanitarios para el muestreo. https://dof.gob.mx/nota_detalle.php?codigo=2081772&fecha=12/07/2005
9. Shapiro, S. S., & Wilk, M. B. (1965). An analysis of variance test for normality (complete samples). *Biometrika, 52*(3/4), 591-611. https://doi.org/10.2307/2333709
10. Soto, P. J. L. (2013). Contraste de hipótesis. Comparación de más de dos medias independientes mediante pruebas no paramétricas: Prueba de Kruskal-Wallis. *Revista Enfermería del Trabajo, 3*(4), 166-171. https://bit.ly/3e2VEmy
11. DOF (2021). NOM-127-SSA1-2021. Agua para uso y consumo humano. Límites permisibles de la calidad del agua. https://www.dof.gob.mx/nota_detalle.php?codigo=5650705&fecha=02/05/2022#gsc.tab=0
12. Criterios Ecológicos de Calidad del Agua, § CE-CCA-001/89. (1989). https://www.dof.gob.mx/nota_detalle.php?codigo=4837548&fecha=13/12/1989#gsc.tab=0
13. World Health Organization (2006). Guidelines for Drinking-water Quality. https://apps.who.int/iris/bitstream/handle/10665/43428/9241546964_eng.pdf
14. Sánchez, J. A., Álvarez, T., Pacheco, J. G., Carrillo, L., & González, R. A. (2016). Calidad del agua subterránea: acuífero sur de Quintana Roo, México. *Tecnología y ciencias del agua, 7*(4), 75-96.
15. Rauf, A., Nasir, A., & Rashid, H. (2021). Assessment of groundwater quality in tehsil gojra by using geographical information system. https://doi.org/10.26480/ecr.02.2021.54.57
16. Vijayakumar, C. R., Balasubramani, D. P., & Azamathulla, H. M. (2022). Assessment of groundwater quality and human health risk associated with chromium exposure in the industrial area of Ranipet, Tamil Nadu, India. *Journal of Water, Sanitation and Hygiene for Development, 12*(1), 58-67. https://doi.org/10.2166/washdev.2021.260
17. Otomewo, J. N. (2022). Evaluation of heavy metals' contamination of ground water and soil around Mbodo-Aluu dumpsites for sustainable agriculture. *Faculty of Natural and Applied Sciences Journal of Scientific Innovations, 3*(2), 71-76. https://fnasjournals.com/index.php/FNAS-JSI/article/view/34
18. Lanjwani, M. F., Khuhawar, M. Y., Jahangir Khuhawar, T. M., Lanjwani, A. H., Jagirani, M. S., Kori, A. H., Rind, I. K., Khuhawar, A. H., & Muhammad Dodo, J. (2020). Risk assessment of heavy metals and salts for human and irrigation consumption of groundwater in Qambar city: a case study. *Geology, Ecology, and Landscapes, 4*(1), 23-39. https://doi.org/10.1080/24749508.2019.1571670
19. Olías, M., Cerón, J., & Fernández, I. (2005). Sobre la utilización de la clasificación de las aguas de riego del US Laboratory Salinity (USLS). *Geogaceta, 37*(3), 111-113. https://rabida.uhu.es/dspace/handle/10272/8899

Antioxidant activity and sensory acceptability of whey protein-based smoothie beverages made from mango (*Mangifera indica* L.) cv Haden and strawberry (*Fragaria* × *ananassa* Duch.) cv Festival

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ABSTRACT

Objective: To assess the effect of different whey proteins levels on the physicochemical, antioxidant activity, and sensory acceptability of smoothie beverages made from mango and strawberry.

Design/methodology/approach: Twenty-four formulations were evaluated (type of fruit and concentration level of whey protein).

Results: Adding of whey protein to smoothies composed of mango and strawberry increased the protein content and antioxidant activity by 2-2.5-fold compared to control smoothies' samples without whey protein. Sensory analysis showed that in terms of overall acceptance, all produced smoothies were considered very acceptable by the panelists.

Limitations on study/implications: Smoothies enriched with whey proteins can be a good new food product that incorporated nutritional and functional compounds into the human diet.

Findings/conclusions: Smoothies produced from mango and strawberry fruits enriched with whey proteins can be considered valuable products as source of bioactive compounds and from sensory points of view.

Keywords: smoothie, antioxidant activity, whey protein, acceptability

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INTRODUCTION

Currently, consumers are concerned about topics related with their health, correct nutrition, and well-being because the modern life is characterized by a stressful life-style, vegetable and fruit-poor diet, and lack of physical activity (Ali & Ali, 2020; Bailey *et al.*, 2020). The above triggered oxidative stress in the body of consumers because there is an excessive amount of reactive oxygen species in their cells, which contributes directly

to developing inflammatory diseases, ischemic diseases, certain cancers, and accelerated aging (Checa & Aran, 2020).

In this context, functional foods have emerged as a complementary alternative in order to improve the lifestyle of consumers. Besides, in recent years consumer trends are orientated to ready-to-eat and/or reformulation of typical food products to increase their nutritional value (Kidoń & Uwineza, 2022). One of foods than can fulfill these requirements and demands are the smoothies, which can be enriched with some bioactive compound with health-promoting properties.

Typically, smoothies are semi-liquid beverages resulting from the blend of fruit- and/or vegetable-based products (*e.g.*, juice and/or puree) with the possible incorporation of milk or yogurt (Kidoń & Uwineza, 2022; Uzodinma *et al.*, 2020). Besides, other ingredients can be added such as species, ice, water, sugar or sweeteners (Uzodinma *et al.*, 2020). In the functional food field, smoothies have been generated by the addition of fruit seeds and peels or by using raw materials rich in phytochemicals (Baiano, Mastromatteo, & Del Nobile, 2012; Fernandez *et al.*, 2020; Picouet *et al.*, 2016; Saini & Sharma, 2020; Tkacz *et al.*, 2021).

Whey proteins, once considered waste from cheese manufacture, have proven to be a source of bioactive components, and when used in functional foods can potentially lead to prevention of lifestyle diseases (Sharma, 2019). Besides, those proteins can provide to beverages some tecnofunctional properties such as foaming, emulsifying, and solubility, which contributes to the increase in the viscosity, texture, and taste of smoothie that influences the organoleptic characteristics of the beverage (Kristensen *et al.*, 2021). Therefore, the aim of this study was the development of new smoothie formulations based on fruits and whey protein, as well as to investigate their sensory characteristics and antioxidant activity of the obtained products.

MATERIALS Y METHODS

Low-fat milk (LALA®), natural yogurt (LALA®), mangoes, and strawberries were obtained from a local supermarket in Hermosillo, Sonora, Mexico. The fruits used in the present study were mango (*Mangifera indica* L.) cv Haden and strawberry (*Fragaria* × *ananassa* Duch.) cv Festival. The fruits were selected with an optimum degree of maturity (intense color, characteristic aroma, and firmness). A commercial unflavored whey protein isolate (Isopure, 86.2% protein content) was obtained from General Nutrition Center (GNC, Mexico). All chemicals used were analytical or gradient grade (for HPLC) purity and provided by Sigma Aldrich (St. Louis, MO, USA) and Thermo Fisher Scientific (Waltham,MA, USA).

Preparation of smoothies-type mango and strawberry beverages

Four different formulations were prepared in triplicate for each fruit (mango and strawberry), obtaining a total of 24 experimental units. The smoothie formulation was prepared by mixing milk (250 mL), natural yogurt (250 mL), fruit (20 g), chipped ice (250 g), and different concentrations of whey protein (0, 10, 20, 30% w/v). The ingredients were homogenized using an Osterizer blender model 450–10 (Sunbeam

Mexicana, S.A. de C.V., Mexico). Smoothie's samples were stored under refrigerated conditions at 4 °C in sterile bottles until analysis in the same day.

Physicochemical analysis

Titratable acidity (AOAC 947.05, 1997), pH (AOAC 981.12, 1997), total protein (Kjeldahl, AOAC 920.123,1997) and total fat (Babcock, AOAC 989.04,1997) contents were determined according to the methods described in the Association of Official Analytical Chemist.

Determination of antioxidant activity

Samples were centrifugated at 12,000 g (Bechman J2-21, Fullerton, USA) during 40 min at 4 °C. The supernatant of each sample was collected and was used to determine the antioxidant activity through 2,2'-azinobis(3-ethylbenzothiazoline)-6-sulphonic acid (ABTS) assay according to the methodology reported by Re *et al.* (1999). Briefly, the ABTS radical was prepared by mixing a solution of ABTS reagent (7 mM) with potassium persulfate (2.45 mM). The mixture was kept in the dark during 16 h at 30 °C. The ABTS working solution was prepared by dilution of the ABTS radical stock solution with PBS (5 mM, pH 7.2) until reached an optical density on 0.7 ± 0.02 (734 nm). Next, 10 μL of sample was mixed with 1 mL of ABTS working solution and the optical density was recorded at 734 nm after 7 min. Trolox (6-hydroxy-2,3,7,8-tetramethylchroman-2-carboxylic acid; Sigma-Aldrich) was used as a standard to prepare a reference curve (0 to 3.5 mM in PBS) to compare those readings obtained by the samples. The results were expressed as millimoles of Trolox equivalents.

Sensory evaluation

Samples were evaluated using a 5-point hedonic scale (5-like very much; 4-like slightly; 3-neither like nor dislike; 2-dislike slightly; 1-dislike very much). The assessment included the following quality attributes: aroma, appearance, acidity, taste, and overall acceptance. The sensory evaluation was conducted by a group of 27 semi-trained panelists (11 women and 16 men, aged 20-45) with formal sessions training in sensory evaluation. To qualify, panelists had to be non-smokers, and had to have no allergy to any of the smoothie ingredients. The panelists were selected based on their interest and skill in sensory assessment and were instructed in the evaluation of the parameters before described. The training was adapted to achieve understanding of the sensory parameter measurement scope and sensory borderlines, and this was achieved via individual testing, followed by group discussions. The sensory tests of each sample were conducted properly in a sensory assessment room equipped with separated cabinets and individual lightning at 20 ± 2.0 °C. The samples were coded with three-digit numbers, served randomly to the panelists, and the data was analyzed.

Statistical analysis

All data were analyzed by one-way analysis of variance (ANOVA). Differences among means were determined at 5% level of significance by the Tukey's test, for physicochemical parameters and antioxidant activity, or Fisher's test, for sensory evaluation data. All analyses were performed using the NCSS software version 2007 (NCSS Statistical software, Kaysville, UT, USA).

RESULTS Y DISSCUSION

Physicochemical parameters

Titratable acidity, pH, total protein, and total fat contents are presented in Table 1. The results showed that the titratable acidity of both type of smoothies (mango and strawberry) ranged between 3.6 and 4.8, and that pH ranged between 4.7 and 5.03. The pH and titratable acidity were different due to different ingredient ratios of whey protein used in the formulation of smoothies. In this context, it was observed a progressive increase of pH and titratable acidity according to the concentration of whey protein in the produced smoothies regardless of if was made with mango or strawberry. This behavior could be explained by the fact that whey protein can increase the pH due to their buffering capacity (Sodini, Mattas, & Tong, 2006). Some physicochemical parameters are important features in foods, such is the case of pH and acidity because are important for sensory characteristic and for the prevention of microbial spoilage. It has been described that some fruit juices could successfully replace of current artificial acidity regulators, particularly in functional food development (Koss-Mikołajczyk *et al.*, 2015). The values found in the present study were in accordance with those reported in the literature. For example, a smoothie made with pineapple, watermelon, banana, and coconut milk had a pH about 4.5 (Uzodinma *et al.*, 2020), whereas other similar smoothie made with banana, pumpkin, and purple carrot had a pH of 4.45, while a smoothie made with mango showed a pH of 4.57 (Balaswamy *et al.*, 2013).

On the other hand, the incorporation of whey protein in the smoothie had a clearly direct effect on their protein content, namely, as the amounts of whey protein increased, the content of the proteins on the smoothies also increased. In both type of smoothies (mango and strawberry), the samples with 30% of whey protein added, showed the highest protein content (4.92-4.94%), while control samples showed lowest protein content (1.75-1.90%). The increase of protein in the sample with 30% of protein whey added was of 2.6-fold compared to control sample. Our results are according to similar studies reported in the literature (Camargo *et al.*, 2018; Maravić *et al.*, 2022; Zavareze, Morales, & Salas-

Table 1. Physicochemical characteristics of smoothies made from mango and strawberry with different concentration of whey proteins.

Parameters	Mango				Strawberry			
	0%	10%	20%	30%	0%	10%	20%	30%
Protein (%)	1.75±0.28[a]	2.38±0.06[b]	4.14±0.09[c]	4.92±0.45[d]	1.90±0.12[a]	2.41±0.05[b]	4.17±0.32[c]	4.94±0.16[d]
pH	4.70±0.09[a]	4.94±0.14[b]	4.97±0.12[c]	5.03±0.25[d]	4.82±0.23[a]	4.91±0.11[b]	5.03±0.29[c]	5.01±0.08[d]
Titratable acidity (g/L of lactic acid)	3.62±0.34[a]	3.94±0.37[b]	4.60±0.28[c]	4.81±0.11[d]	3.60±0.37[a]	4.00±0.24[b]	4.09±0.18[c]	4.70±0.13[d]

* Values are mean of triplicate determinations. Different letters indicate statistical difference among formulations with different level of whey proteins for each parameter and type of smoothie. Fat was evaluated but was not included in the table due to did not show changes for all samples (ca. 3% of fat).

Mellado, 2010), where more whey protein is added to a food product formulation, the higher is the progressive protein increase. This is important because whey protein is a nutritious product that contain biologically active superior proteins (Musina *et al.*, 2018). In a study realized by Corgneau *et al.* (2019) found that among various commercial protein supplements, the whey protein showed highest in vitro digestibility (ca. 90%), while egg white powder showed less digestibility (ca. 81%). In addition to their excellent digestibility, whey proteins showed outstanding bioavailability and essential amino acid contents that meeting the human needs (McGregor & Poppitt, 2013). Then, the addition of whey protein increases the nutritional quality of smoothie's samples. In contrast, the content of fat did not show changes at different levels of the incorporation of whey protein in both type of mango and strawberry smoothie's samples (ca. 3%).

Antioxidant activity

It is known that the antioxidant activity of bioactive compounds can help deal with the effects of the pathological condition called oxidative stress, which is a disturbance in the balance between the production of reactive oxygen species (free radicals) and antioxidant defenses. Oxidative stress contributes directly to developing inflammatory diseases, ischemic diseases, certain cancers, and accelerated aging (Checa & Aran, 2020). Antioxidant activity data showed that all smoothies formulations made from mango and strawberry with different levels of why proteins had antioxidant proprieties in different extents (Table 2).

It has been observed that as the amounts of whey protein increased, the antioxidant activity on both type of smoothies also increased, being statically different ($P>0.05$) the formulations with highest content of whey protein (20 and 30%). The antioxidant activity of the smoothies with 30% of whey protein added were ca. 2-2.5-fold higher than the control smoothie without whey protein. In related works, it has been reported the antioxidant activity of different formulations of smoothies. The results of our study was higher than those reported by Yassin *et al.* (2020), who evaluated the antioxidant activity of 17 commercial smoothies marketed in Brazil. Their results showed that the antioxidant activity ranged from 1.487 to 7.333 mM of Trolox equivalents for DPPH method and from 1.819 to 9.801 mM of Trolox equivalents for ABTS method. Similarly, Nowicka, Wojdyło, and Samoticha

Table 2. Antioxidant activity (mM of Trolox equivalents) of smoothies made from mango and strawberry with different concentration of whey proteins.

Whey protein (%)	Smoothies	
	Mango	Strawberry
0	17.54 ± 1.89^{a}	14.15 ± 1.24^{a}
10	19.12 ± 1.02^{a}	14.88 ± 1.00^{a}
20	23.93 ± 2.03^{b}	27.30 ± 2.32^{b}
30	33.36 ± 0.79^{c}	33.92 ± 0.93^{c}

*Values are mean of triplicate determinations. Different letters indicate statistical difference among formulations with different level of whey proteins for each type of smoothie.

(2016) reported the antioxidant activity of 13 smoothies based on different combinations of *Prunus* fruits (*e.g.*, cherry (*Prunus cerasus* L.) cv Lutowka, peach (*Prunus persica* L.) cv Hardow beauty, apricot (*Prunus americana* L.) cv Miodowa, and plum (*Prunus* L.) cv Promis). The authors found that the antioxidant activity ranged from 2.35 to 6.81 mM of Trolox/100 g sample for ABTS method and from 1.33 to 4.23 mM of Trolox/100 g sample for ORAC method. For the above studies, it is possible that the differences are due to the proportion and constituents of each beverage not being uniform, justifying the variation. Likewise, a vegetable smoothie made from Beet plants (*Beta vulgaris* L. cv Conditiva) showed an antioxidant capacity of 219.2 mM of Trolox/kg sample for FRAP and 28.9 mM of Trolox/kg sample for DPPH (Fernandez *et al.*, 2020). In a study realized by Keenan *et al.* (2010) reported values of antioxidant activity of 253.6 mg of Trolox equivalents/100 g dry sample of a smoothie made from a blend of strawberry, apple, banana, and orange. Besides, the authors treated the smoothie sample thermally way, which increased their antioxidant activity by 15%.

Because methodologies for evaluating antioxidant activity may vary in different aspects, such as the assay used and the way to express the results, comparison of results from different studies is as difficult as it is to distinguish only one mechanism or compound involved in the antioxidant activity (Alam, Bristi, & Rafiquzzaman, 2013).

Sensory evaluation

In the sensory evaluation of smoothies made from mango and strawberry (Table 3), it was not found significant differences ($P>0.05$) in the most formulations for the follow attributes: aroma, appearance, taste, and overall acceptance; however, the acidity was the only one which showed significant difference in the smoothies from mango formulated with high content of whey proteins (20% and 30%) compared with those formulated with lowest content of protein whey. Besides, highest sensory score in all attributes was found in smoothies made from strawberry compared with those showed in the smoothies made from mango.

Furthermore, most formulations scored above 3.5 for overall acceptance, which represent the equivalent to "like slightly". Thus, the also showed that in terms of overall acceptance, all produced smoothies were considered very acceptable by the panelists.

Table 3. Sensory analysis of smoothies made from mango and strawberry with different concentration of whey proteins.

Attributes	Mango				Strawberry			
	0%	10%	20%	30%	0%	10%	20%	30%
Aroma	3.77^{a}	3.85^{a}	3.63^{a}	3.60^{a}	3.74^{a}	3.96^{ab}	3.48^{b}	3.88^{ab}
Appearance	3.66^{a}	3.70^{a}	3.59^{a}	3.61^{a}	3.51^{a}	3.85^{a}	3.55^{a}	3.81^{a}
Acidity	3.96^{a}	3.96^{a}	3.25^{b}	3.25^{b}	3.70^{a}	3.81^{a}	3.51^{a}	3.70^{a}
Taste	3.77^{a}	3.48^{ab}	3.00^{b}	3.25^{ab}	4.00^{a}	4.14^{ab}	3.51^{b}	3.85^{ab}
Overall acceptance	4.00^{a}	3.74^{ab}	3.37^{b}	3.54^{ab}	3.96^{a}	3.9^{a}	3.55^{a}	3.88^{a}

Values are mean of evaluators. Different letters indicate statistical difference among formulations with different level of whey proteins for each attribute and type of smoothie.

CONCLUSIONS

Smoothies produced from mango and strawberry fruits enriched with whey proteins can be considered valuable products as source of bioactive compounds and from sensory points of view. Incorporation of whey proteins into mango and strawberry smoothies significantly enriched protein content of the product with the concomitant increase in their antioxidant activity. Besides, the sensory panelists approved of all the smoothies formulated. However, more research is needed to check changes of bioactive compound content during the storage of smoothies.

REFERENCES

Alam, M. N., Bristi, N. J., & Rafiquzzaman, M. (2013). Review on *in vivo* and *in vitro* methods evaluation of antioxidant activity. *Saudi Pharmaceutical Journal, 21*(2), 143-152. doi:https://doi.org/10.1016/j.jsps.2012.05.002

Ali, T., & Ali, J. (2020). Factors affecting the consumers' willingness to pay for health and wellness food products. *Journal of Agriculture and Food Research*, 2, 100076. doi:https://doi.org/10.1016/j.jafr.2020.100076

Baiano, A., Mastromatteo, M., & Del Nobile, M. A. (2012). Effects of cultivar and process variables on dynamic-mechanical and sensorial behavior of value-added grape-based smoothies. *Molecules, 17*(10), 11421-11434. doi:https://doi.org/10.3390/molecules171011421

Bailey, J. M., Clinton-McHarg, T. L., Wye, P. M., Wiggers, J. H., Bartlem, K. M., & Bowman, J. A. (2020). Preventive care for physical activity and fruit and vegetable consumption: a survey of family carer expectations of health service delivery for people with a mental health condition. *BMC Health Services Research, 20*(1), 201. doi:https://doi.org/10.1186/s12913-020-5059-0

Balaswamy, K., Prabhakara Rao, P. G., Nagender, A., Narsing Rao, G., Sathiya Mala, K., Jyothirmayi, T., Math, R. G., Satyanarayana, A. (2013). Development of smoothies from selected fruit pulps/juices. *International Food Research Journal, 20*(3).

Camargo, L. R., Silva, L. M., Komeroski, M. R., Kist, T. B. L., Rodrigues, C. E., Rios, A. d. O., Silva, M. M., Doneda, D., Schmidt , H. d. O., Oliveira, V. R. (2018). Effect of whey protein addition on the nutritional, technological and sensory quality of banana cake. *International Journal of Food Science & Technology, 53*(11), 2617-2623. doi:https://doi.org/10.1111/ijfs.13857

Checa, J. & Aran, J. M. (2020). Reactive Oxygen Species: Drivers of Physiological and Pathological Processes. *Journal of Inflammation Research*, 13, 1057-1073. doi: https://doi.org/10.2147/JIR.S275595

Corgneau, M., Gaiani, C., Petit, J., Nikolova, Y., Banon, S., Ritié-Pertusa, L., Le, D. T. L., & Scher, J. (2019). Nutritional quality evaluation of commercial protein supplements. *International Journal of Food Science & Technology, 54*(8), 2586-2594. doi:https://doi.org/10.1111/ijfs.14170

Fernandez, M. V., Bengardino, M., Jagus, R. J., & Agüero, M. V. (2020). Enrichment and preservation of a vegetable smoothie with an antioxidant and antimicrobial extract obtained from beet by-products. *LWT-Food Science and Technology*, 117, 108622. doi:https://doi.org/10.1016/j.lwt.2019.108622

Keenan, D. F., Brunton, N. P., Gormley, T. R., Butler, F., Tiwari, B. K., & Patras, A. (2010). Effect of thermal and high hydrostatic pressure processing on antioxidant activity and colour of fruit smoothies. *Innovative Food Science & Emerging Technologies, 11*(4), 551-556. doi:https://doi.org/10.1016/j.ifset.2010.07.003

Kidoń, M., & Uwineza, P. A. (2022). New smoothie products based on pumpkin, banana, and purple carrot as a source of bioactive compounds. *Molecules, 27*(10). doi:https://doi.org/10.3390/molecules27103049

Koss-Mikołajczyk, I., Kusznierewicz, B., Namieśnik, J., & Bartoszek, A. (2015). Juices from non-typical edible fruits as health-promoting acidity regulators for food industry. *LWT - Food Science and Technology, 64*(2), 845-852. doi:https://doi.org/10.1016/j.lwt.2015.06.072

Kristensen, H. T., Denon, Q., Tavernier, I., Gregersen, S. B., Hammershøj, M., Van der Meeren, P., Dewettinck, K., & Dalsgaard, T. K. (2021). Improved food functional properties of pea protein isolate in blends and co-precipitates with whey protein isolate. *Food Hydrocolloids*, 113, 106556. doi:https://doi.org/10.1016/j.foodhyd.2020.106556

Maravić, N., Škrobot, D., Dapčević-Hadnađev, T., Pajin, B., Tomić, J., & Hadnađev, M. (2022). Effect of sourdough and whey protein addition on the technological and nutritive characteristics of sponge cake. *Foods, 11*(14). doi:https://doi.org/10.3390/foods11141992

McGregor, R. A., & Poppitt, S. D. (2013). Milk protein for improved metabolic health: a review of the evidence. *Nutrition & Metabolism, 10*(1), 46. doi:https://doi.org/10.1186/1743-7075-10-46

Musina, O., Rashidinejad, A., Putnik, P., Barba, F. J., Abbaspourrad, A., Greiner, R., & Roohinejad, S. (2018). The use of whey protein extract for manufacture of a whipped frozen dairy dessert. *Mljekarstvo, 68*(4), 254-271.

Nowicka, P., Wojdyło, A., & Samoticha, J. (2016). Evaluation of phytochemicals, antioxidant capacity, and antidiabetic activity of novel smoothies from selected Prunus fruits. *Journal of Functional Foods*, 25, 397-407. doi:https://doi.org/10.1016/j.jff.2016.06.024

Picouet, P. A., Hurtado, A., Jofré, A., Bañon, S., Ros, J.-M., & Guàrdia, M. D. (2016). Effects of thermal and high-pressure treatments on the microbiological, nutritional and sensory quality of a multi-fruit smoothie. *Food and Bioprocess Technology, 9*(7), 1219-1232. doi:https://doi.org/10.1007/s11947-016-1705-2

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine, 26*(9), 1231-1237. doi:https://doi.org/10.1016/S0891-5849(98)00315-3

Saini, D., & Sharma, S. (2020). Utilization of sand pear and orange peel to develop value added smoothie. *Pharma Innovation Journal, 9*(6), 200-202.

Sharma, R. (2019). Chapter 17 - Whey proteins in functional foods. In H. C. Deeth & N. Bansal (Eds.), Whey Proteins (pp. 637-663): Academic Press.

Sodini, I., Mattas, J., & Tong, P. S. (2006). Influence of pH and heat treatment of whey on the functional properties of whey protein concentrates in yoghurt. *International Dairy Journal, 16*(12), 1464-1469. doi:https://doi.org/10.1016/j.idairyj.2005.03.014

Tkacz, K., Wojdyło, A., Turkiewicz, I. P., & Nowicka, P. (2021). Anti-diabetic, anti-cholinesterase, and antioxidant potential, chemical composition and sensory evaluation of novel sea buckthorn-based smoothies. *Food Chemistry*, 338, 128105. doi:https://doi.org/10.1016/j.foodchem.2020.128105

Uzodinma, E. O., Mbaeyi-Nwaoha, I. E., Onwurafor, E. U., & Chidinma Ezinne, O. (2020). Influence of pasteurization on the quality of pineapple, watermelon and banana pulps-based smoothie flavoured with coconut milk. *American Journal of Food Science and Technology, 8*(3), 99-105. doi:https://doi.org/10.12691/ajfst-8-3-3

Yassin, L. S., Zielinski, A. A. F., Alberti, A., Los, P. R., Demiate, I. M., Simões, D. R. S., & Nogueira, A. (2020). An overview of Brazilian smoothies: from consumer profile to evaluation of their physicochemical composition, bioactive compounds, antioxidant activity and sensory description. *Journal of Food Bioactives, 10*(0). doi:https://doi.org/10.31665/JFB.2020.10223

Zavareze, E. R., Moraes, K. S., & Salas-Mellado, M. M. (2010). Technological and sensory quality of cakes produced with milk whey. *Food Science and Technology*, 30, 100-105. doi: https://doi.org/10.1590/S0101-20612010000100015

Colegio de Postgraduados

Prospects in Vanilla (*Vanilla planifolia* Andrews) production in Mexico in relation to temperature fluctuations

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ABSTRACT

Objective: To establish a time series model correlating vanilla production with temperature variation to forecast vanilla production in Mexico.

Design/methodology/approach: Data on vanilla production in Mexico (Tons) and the annual average temperature were obtained for the period 1985-2020. An ARIMA model was constructed; Granger causality test was conducted to determine the effect of temperature on vanilla production, in addition to evaluating the orthogonal response of the model. A forecast for vanilla production was made for the period 2020-2040.

Results: ARIMA (1,1,1) model was found, and the influence of temperature on vanilla production was determined. Both thermal variation and the production of the last three years determine current production. A reduction in the quantity of tons of vanilla produced in the coming years is expected. It is considered that this cultivation is highly sensitive to sudden increases or decreases in temperature.

Limitations on study/implications: Vanilla cultivation is sensitive to temperature variation; therefore, in the face of climate change, it is considered necessary to take a series of actions in the present. These actions encompass a genetic perspective, new cultivation methods and locations, as well as technological investment.

Findings/conclusions: Vanilla production is influenced by temperature variation and is sensitive to sudden increases or declines. If actions are not taken in the present, a reduction in the national production of vanilla in Mexico is expected due to climate change.

Keywords: ARIMA model, Climate change, Crops, Orchidaceae, Time series.

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INTRODUCTION

The threat of climate change has caused concern in recent years, as essential climatic factors for crop growth such as precipitation and temperature will be severely affected, and thus they will impact agricultural production (Armenta-Montero *et al.*, 2022; Hatfield and Prueger, 2015). Although the effects of climate change on crop production may vary from one region to another, it is expected that the predicted changes will have far-reaching impacts, especially in countries with tropical zones (Heino *et al.*, 2019). In the face of this possible future scenario, the consequences can be profound for subsistence farmers located in fragile environments, where significant changes in productivity are expected (Raza *et al.*, 2019).

One of the crops that will be affected by climate change is vanilla (Barreda-Castillo *et al.*, 2023a). Vanilla (*Vanilla planifolia* Andrews) is an orchid of economic interest because its fruits are highly valued in the food and aromatic industry (Hernández-Hernández, 2018a), in addition to medicinal properties (Teoh, 2019). Despite its economic importance, this species is classified as "subject to special protection" (SEMARNAT, 2010), and it is also protected worldwide by the Convention on International Trade in Endangered Species of Wild Flora and Fauna, and the International Union for Conservation of Nature Red List (Armenta-Montero *et al.*, 2022; IUCN, 2022). Due to the plants have been propagated asexually, genetic erosion has occurred, and the cultivation has become vulnerable to several scenarios, including climate change (Hernández-Hernández, 2018b).

The cultivation of vanilla in Mexico has experienced fluctuations throughout its history. In recent times, there was a maximum production of 637 tons in 2007, and a minimum production of 10 tons in 1979 (Figure 1). However, México used to be the world's leading vanilla producer in the period between 1870 and 1910, which is also called "the era of mass production of vanilla", where Mexico exported large tons of this fruit to the world (Kouri, 2000; Lubinsky *et al.*, 2018), to become nowadays a minor producer, surpassed by other countries such as Madagascar and Indonesia, for example (Grisoni and Nany, 2021). There is a relationship between the increase in temperature during flowering season and a subsequent loss of fruit-forming capacity, causing significant economic losses (Hernández-Hernández and Lubinsky, 2011). Furthermore, it should be considered that the traditional way of cultivating it in areas where temperatures have risen will not be able to meet the future needs of this crop (Armenta-Montero *et al.*, 2022; Barreda-Castillo *et al.*, 2023a).

Regarding the uncertainty of climate change, prospective studies become necessary, as it has been done in crops such as beans (Medina-García *et al.*, 2016), as well as in banana (Hamjah, 2014), corn (Verma *et al.*, 2012), cotton (Debnath *et al.*, 2013), sugarcane (Suresh and Priya, 2011), wheat (Iqbal *et al.*, 2005), among others. An alternative is the use of the

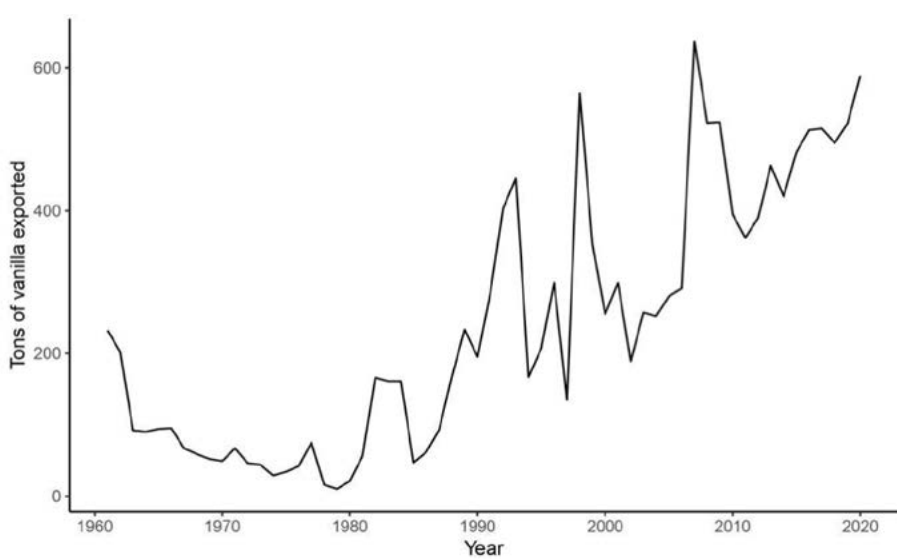

Figure 1. Historical vanilla production in Mexico, during the period 1960-2020. Figure constructed from data reported by UNdata (2023).

ARIMA model (Auto Regressive Integrated Moving Average), which is useful in time series exhibiting seasonality (Fattah *et al.*, 2018; Wang *et al.*, 2020), as is the case with vanilla, as the majority of the production is obtained only in certain months of the year (Hernández-Hernández, 2018a). Even though there is a first approach to predicting the behavior of vanilla production in Mexico (Luis-Rojas *et al.*, 2020), that model did not consider external factors such as thermal oscillation, which can cause stochastic events in this fragile crop. Therefore, the objective of this study was to establish a time series model relating the national vanilla export and the variation in Mexico's annual average temperature to forecast future production values, in order to propose actions in the present.

MATERIALS AND METHODS

Data on the average annual temperature (°C) of Mexico was obtained for the period between 1985 and 2020 from information provided by the National Water Commission, available through CONAGUA (2023). To ascertain the historical vanilla production in Mexico, export records (tons) published by the Food and Agriculture Organization of the United Nations were consulted, avaliable from UNdata (2023). Official records were considered from the year 1985 to 2020.

To determine if the vanilla production data correspond to a stationary series, Dickey-Fuller Test ($p=0.05$) was carried out. Possible values of MA (q) and AR (p) were determined using the autocorrelation function (ACF) and partial ACF, respectively. To determine the effect that temperature will have on the future production of vanilla in Mexico in the period 2020-2040, a multivariate time series analysis using an autoregressive integrated moving average (ARIMA) model was employed (Tsay, 2014).

Ljung-Box test was performed ($p=0.05$) to corroborate that the residuals were white noise (Hassani and Yeganegi, 2020). Additionally, a Granger causality test (Granger, 1969) was conducted to verify the effect of temperature on vanilla production. The orthogonal response of the model was evaluated, in search of sensitivity to stochastic events.

The statistical analysis was performed using R 3.6 software (R core team, 2017), with astsa (Stoffer and Poison, 2023), forecast (Hyndman and Khandakar, 2008), seasonal (Sax and Eddelbuettel, 2018), TSA (Chan and Ripley, 2022), tseries (Trappletti and Hornik, 2023), urca (Pfaff, 2008), and vars (Pfaff, 2008) packages.

RESULTS AND DISCUSSION

It was determined that vanilla production data in Mexico correspond to a stationary series, with a lag order of 3 (Dickey-fuller$=-3.8238$, $p<0.05$). Thus, vanilla production is dependent on temperature variations and agricultural management over the three previous years. This could be related to the three years of waiting that producers make before obtaining flowering, and therefore it may determine future vanilla production (Iftikhar *et al.*, 2023).

Partial ACF analysis exhibited possible AR (p) values of 0 and 1 (Figure 2), whereas ACF analysis exhibited possible MA (q) values of between 1 and 12 (Figure 2). In order to select the best model that described the behavior of the data, the auto.arima function was used (Hyndman and Khandakar, 2008), obtaining the model ARIMA (1,1,1) ($\sigma^2=8884$, log

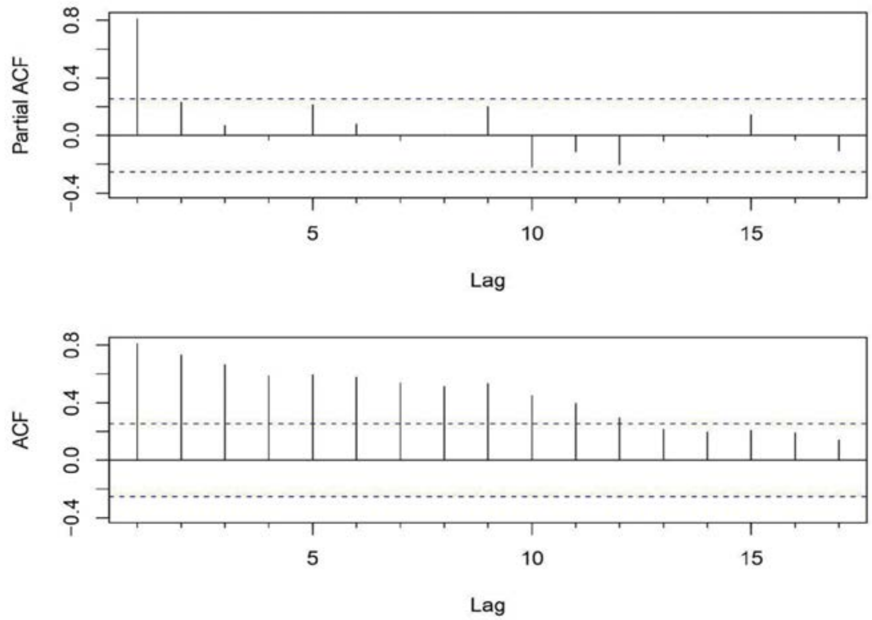

Figure 2. Partial autocorrelation function (ACF) and ACF estimated for ARIMA (1,1,1) model.

likelihood=−350.7, AIC=709.4, BIC=717.71). It was confirmed that the model residuals were white noise (D. F.=20, χ^2=25.777, p=0.17).

The model we found was similar to the one reported by Luis-Rojas *et al.* (2020), however, they did not consider external factors such as temperature, as we did. In contrast, we found the influence of temperature on vanilla production through Granger causality test (D.F.=32, F=13.245, p<0.01). It was confirmed that the model only operated in one direction, this means, no influence of vanilla production on temperature fluctuations was found (D.F.=32, F=13.245, p=0.74).

The equation defining the model is expressed in the following formula:

$$X_t = 101.91331 X_{1t-1} + 0.03847 X_{2t-1} + 30.99266 X_{1t-2} + 0.01684 X_{2t-2}$$
$$+ 58.45541 X_{1t-3} - 0.017137 X_{2t-3} - 3665.309 + a_{1t}$$

Where X_t refers to the predicted value of vanilla produced, 1_{t-n} to the value dependent on the quantity of vanilla tons, the coefficient 2_{t-n} to temperature variations, and a_{1t} to the model error (D.F.=26, F=5.832, p<0.05). The proposed model was compared against the predicted values for 2020-2022 with respect to the values reported by Gobierno de México (2023). In 2020, a difference of 11.72 tons was observed, in 2021 of 2.41 tons, and in 2022 of 4.74 tons (Figure 3).

Based on the aforementioned equation, the production value of vanilla tons in Mexico was estimated, along with its minimum and maximum values. Contrary to the model proposed by Luis-Rojas *et al.* (2020), our model allowed us to predict vanilla production for the period 2020-2040. The predicted values are expressed in Table 1.

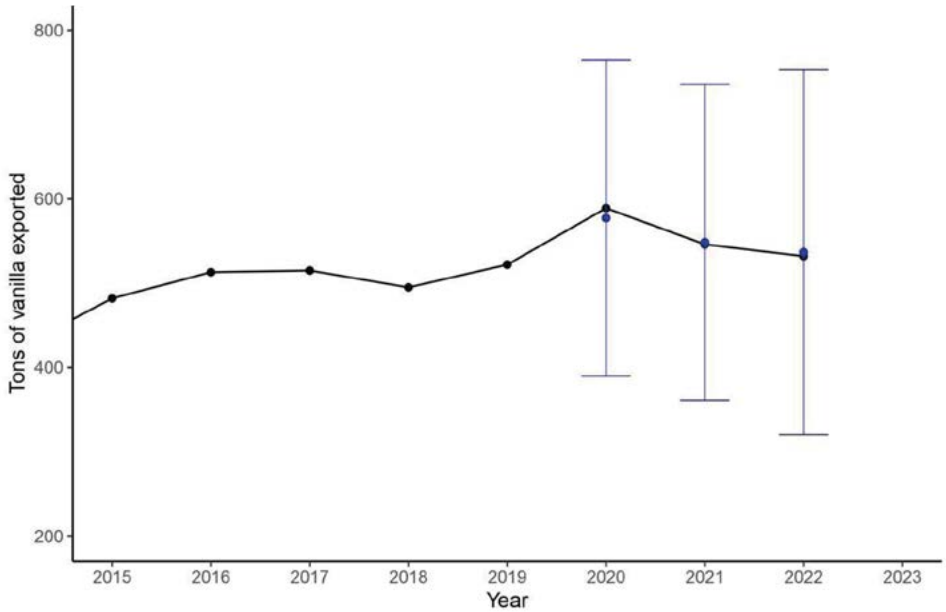

Figure 3. Comparison of the values reported by the ARIMA model (1,1,1) respect to the last three values of vanilla production (tons) in Mexico. Black: tons of vanilla exported in México, according to UNdata (2023) and Gobierno de México (2023). Blue: predicted vanilla tons.

Table 1. Expected vanilla production in Mexico (tons) for the period 2020-2040. (CI=95%).

Year	Forecast	Lower	Upper
2020	577.2763	389.79600	764.7566
2021	548.4151	360.93484	735.8954
2022	536.7486	320.40706	753.0901
2023	533.3949	300.91853	765.8713
2024	532.4309	287.05021	777.8115
2025	532.1537	275.08378	789.2237
2026	532.0741	263.97834	800.1698
2027	532.0512	253.40812	810.6942
2028	532.0446	243.25095	820.8382
2029	532.0427	233.44661	830.6388
2030	532.0422	223.95626	840.128
2031	532.042	214.75025	849.3337
2032	532.042	205.80409	858.2798
2033	532.0419	197.09685	866.987
2034	532.0419	188.61031	875.4736
2035	532.0419	180.32848	883.7554
2036	532.0419	172.23723	891.8466
2037	532.0419	164.32398	899.7599
2038	532.0419	156.57747	907.5064
2039	532.0419	148.98759	915.0963
2040	532.0419	141.5452	922.5387

Similar to Luis-Rojas *et al.* (2020) model, vanilla production will decrease, however, in this forecast study it is considered that the future average annual production of vanilla in Mexico will be around 532 tons. Even the highest values (mean plus confidence interval) predicted are still lower than the current production of the main producing countries (Borbolla-Pérez *et al.*, 2017). It is unlikely that Mexico will once again be one of the main vanilla producing countries in the world, however, mexican vanilla is still considered as the best quality vanilla produced worldwide (Ellestad *et al.*, 2022; Ranadive, 2018), which is why it is suggested to focus efforts on continuing to produce quality vanilla instead of quantity.

Regarding the orthogonal response, the model showed susceptibility to stochastic events (Figure 4), which implies that any abrupt alteration in temperature, national vanilla production would be seriously affected. This is similar to what has been reported in the past, since in 1955 a stochastic event (hurricanes Hilda, Gladys and Janet) almost caused the total loss of vanilla production in Mexico, as well as a second event in 1961 (there was an extremely cold period) in which vanilla cultivation was almost lost nationwide (Suárez-Barrios, 2016; Vera-Cortés, 2007). Due to climate change, global temperatures are expected to increase between 3 to 5 °C (Luo *et al.*, 2020), although some predictions are less conservative and predict an increase of up to 11 °C (Reddy *et al.*, 2017). This increase in temperature will have negative consequences for the vanilla crop, since it has been observed that prolonged exposure to 32 °C (an increase of 7 °C above the optimum) reduces the production of roots and shoots, while exposure to 35 °C (an increase of up to 10 °C) is inhibited (Barreda-Castillo *et al.*, 2023a); furthermore, it has been observed that exposure to temperatures equal to or greater than 32 °C affects the viability of pollen, as well as the formation and maintenance of fruits (Hernández-Hernández, 2018; Iftikhar *et al.*, 2023; Menchaca-García, 2018). The susceptibility of vanilla culture may not be limited to temperature or just to Mexico, since another vulnerability scenarios have been observed, like in Puerto Rico (Bayman, 2018), or Indonesia (Pinaria *et al.*, 2010), where the root-stem rot disease caused by *Fusarium oxysporum* f. sp. vanillae significantly reduced the vanilla production.

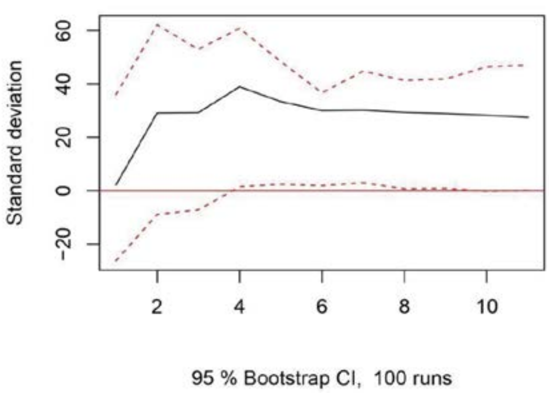

Figure 4. Orthogonal impulse associated with the ARIMA (1,1,1) model, representing the effect of temperature variation on vanilla production in Mexico. The solid line shows the moving averages associated with the model, whereas the dashed lines indicate the response to stochastic variations in temperature.

Due to the observed fragility of the vanilla crop, it is recommended to implement a series of mitigation actions at present. These actions can be considered from the following perspectives:

Genetic: It is important to rescue the use of cultivation with wild species and varieties, which may be more tolerant to new future environmental conditions (Flanagan *et al.*, 2018; Jackson *et al.*, 2007). Species such as *V. pompona* Schiede or *V. odorata* C. Presl, both aromatic vanillas, might be considered for cultivation (Maruenda *et al.*, 2013; Watteyn *et al.*, 2023). In addition, it is necessary to obtain vanilla plants from seed to increase genetic diversity in this crop, and thereby obtain individuals with better responses to biotic and abiotic stress scenarios (Menchaca-García *et al*, 2011; Yeh *et al.*, 2021). Likewise, a hybridization program for species that could provide greater resistance to temperature increases is considered necessary, since these species may also incorporate other aromatic properties for the market (Barreda-Castillo *et al.*, 2023b; Menchaca-García, 2018).

Cultivation Method: Due to its growth on tree supports, the trend of vanilla cultivation in traditional regions has been on citrus monocultures, which have been severely affected by rising temperatures and do not provide sufficient shade from insolation (Estudillo-Hernández *et al.*, 2019). So, management alternatives can be proposed to continue vanilla cultivation. An example of this is establishing cultivation in forest regeneration zones or secondary growth areas (also called "acahual"), where the surrounding vegetation provides shade at different canopy levels and contributes to creating microclimatic zones that help lower the temperature (Hernández-Hernández, 2018a; Ramos-Prado *et al.*, 2023).

Technology: If the trend in vanilla cultivation is to remain in traditional sites (from 0 to 400 meters above sea level), where an increase in temperature has been observed (Armenta-Montero *et al.*, 2022; Soto-Arenas and Dressler, 2009), it will be necessary to establish it using shade nets ranging from 50 to 80%, implemented with sprinkler irrigation systems in order to help to mitigate insolation but, on the other hand, it increases the investment in cultivation (van Noort, 2018).

Cultivation Zones: With the current trend of climate change, there is an observed shift in vanilla cultivation to higher-altitude areas, exceeding even 1000 meters above sea level (Menchaca-García *et al.*, 2019). Thus, it is necessary to implement studies that determine the geographical zones where vanilla plants can thrive, showing appropriate growth, flowering, and fruiting based on environmental tolerance ranges. Likewise, farmer training is essential to consider cultivating vanilla in areas where it has traditionally not been grown (Chambers, 2019).

CONCLUSIONS

Vanilla cultivation follows a seasonal behavior, and in turn is influenced by variations in temperature. The model that describes the behavior of the crop was ARIMA (1,1,1) with three orders of lag, which indicates that the current vanilla production is a function of both the production and the thermal variation of the last three years.

The forecast for vanilla production in Mexico indicates a reduction, compared to current production. Furthermore, the vanilla crop is considered to be susceptible to stochastic variations in temperature. Therefore, it is recommended that actions be taken now regarding genetic improvement, new forms of cultivation, investment in technology, or establishing new cultivation areas, all with the aim of conserving this important crop in Mexico.

Authors contribution: Conceptualization, JMBC and RAMG; Methodology, JMBC; Software, JMBC; Validation, JMBC; Formal analysis, JMBC; Investigation, JMBC and RAMG; Resources, RAMG; Data curation, JMBC;
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REFERENCES

Armenta-Montero, S; Menchaca-García, R; Pérez-Silva, A; Velázquez-Rosas, N. 2022. Changes in the Potential Distribution of Vanilla planifolia Andrews under Different Climate Change Projections in Mexico. IJMS. 14. 2881. Doi: https://doi.org/10.3390/su14052881

Hatfield, JL; Prueger, JH. 2015. Temperature extremes: Effect on plant growth and development. *Weath. Clim. Extr*. 10. 4-10. Doi: https://doi.org/10.1016/j.wace.2015.08.001

Heino, M; Guillaume, JHA; Müller, C; Iizumi, T; Kummu, M. 2019. A multi-model analysis of teleconnected crop yield variability in a range of cropping systems. *Earth Syst. Dyn*. 11. 113-128. Doi: https://doi.org/10.5194/esd-11-113-2020

Raza, A; Razzaq, A; Mehmood, SS; Zou, X; Zhang, X; Lv, Y; Xu, J. 2019. Impact of Climate Change on Crops Adaptation and Strategies to Tackle Its Outcome: A Review. *Plants. 8*(2). 34. Doi: https://doi.org/10.3390/plants8020034

Barreda-Castillo, JM; Menchaca-García, RA; Lozano-Rodríguez, MA. 2023a. Effect of temperature on roots and shoots formation in three vanilla species (Orchidaceae) under controlled conditions. *Trop. And Subtrop. Agroecos*. 26. 1-7. Doi: http://doi.org/10.56369/tsaes.4855

Hernández-Hernández, J. Mexican Vanilla Production. In Handbook of Vanilla Science and Technology, 2nd ed.; Havkin-Frenkel, D, Belanger, F, Eds.; Wiley: New York, United States of America, 2018a; 3-26. Doi: https://doi.org/10.1002/9781119377320.ch1

Teoh, ES. Orchids as aphrodisiac, medicine or food. Springer: Singapore, 2019; 109-130. Doi: https://doi.org/10.1007/978-3-030-18255-7

SEMARNAT. 2010. NORMA Oficial Mexicana NOM-059-SEMARNAT-2010. Protección ambiental-especies nativas de México de flora y fauna silvestres-Categorías de riesgo y especificaciones para su inclusión, exclusión o cambio-Lista de especies en riesgo. Secretaría del Medio Ambiente y Recursos Naturales. http://dof.gob.mx/nota_detalle.php?codigo=5173091&fecha=20/12/2010

IUCN. 2022. Vanilla planifolia. Available in https://www.iucnredlist.org/species/103090930/172970359

Hernández-Hernández, J. Vanilla diseases. In Handbook of Vanilla Science and Technology, 2nd ed.; Havkin-Frenkel, D, Belanger, F, Eds.; Wiley: New York, United States of America, 2018b; 181-190. Doi: https://doi.org/10.1002/9781119377320.ch2

Kouri, EH. 2000. La vainilla de Papantla: agricultura, comercio y sociedad rural en el siglo XlX. *Sig. Hist. 1*(3). 105-130.

Lubinsky, P; Romero-González, G; Heredia, S; Zabel, S. Origins and patterns of Vanilla cultivation in tropical America (1500-1900): No support for an independent domestication of Vanilla in South America. In Handbook of Vanilla Science and Technology, 2nd ed.; Havkin-Frenkel, D, Belanger, F, Eds.; Wiley: New York, United States of America, 2018; 121-146. Doi: https://doi.org/10.1002/9781119377320.ch8

Grisoni, M; Nany, F. 2021. The beautiful hills: half a century of vanilla (***Vanilla planifolia*** Jacks. ex Andrews) breeding in Madagascar. *Gen. Res. Crop Evol*. 68. 1691-1708. Doi: https://doi.org/10.1007/s10722-021-01119-2

Hernández-Hernández, J; Lubinsky, P. Vanilla production in Mexico. In Vanilla, Odoux, E, Grisoni, M, Eds.; New York, United States of America, 2011; 335-347.

Medina-García, G; Ruiz-Corral, JA; Rodríguez-Moreno, VM; Soria-Ruiz J; Díaz-Padilla, G; Zarazúa-Villaseñor, P. 2016. Efecto del cambio climático en el potencial productivo del frijol en México. *Rev. Mex. C. Agr*. 13. 2465-2474.

Hamjah, MA. 2014. Forecasting major fruit crops productions in Bangladesh using Box-Jenkins ARIMA model. *J. Econ. Sust. Dev. 5*(7), 96-107.

Verma, U; Koehler, W; Goyal, M. 2012. A study of yield trends of different crops using ARIMA analysis. *Env. Ecol*. 30. 1459-1463.

Debnath, MK; Bera, K; Mishra, P. 2013. Forecasting area, production and yield of cotton in India using ARIMA model. *J. Sp. Sc. Tech. 2*(1). 16-20.

Suresh, KK; Priya, SRK. 2011. Forecasting sugarcane yield of Tamilnadu using ARIMA models. *Sugar Tech. 13*(1). 23-26. Doi: https://doi.org/10.1007/s12355-011-0071-7

Iqbal, N; Bakhsh, Maqbool, A; Ahmad, S. 2005. Use of ARIMA model for forecasting wheat area and production in Pakistan. *J. Agr. Soc. Sci. 1*(2). 120-122.

Fattah, J; Ezzine, L; Lachhab, A. 2018. Forecasting of demand using ARIMA model. *Int. J. Eng. Buss. Manag*. 10. 1-9. Doi: 10.1177/1847979018808673

Wang, L; Feng, J; Sui, X; Chu, X; Mu, W. 2020. Agricultural product price forecasting methods: research advances and trend. *Brit. F. J. 122*(7). 2121-2138. Doi: https://doi.org/10.1108/BFJ-09-2019-0683

Luis-Rojas, S; Ramírez-Valverde, B; Díaz-Bautista, M; Pizano-Calderón, J; Rodríguez-López, C. 2020. La producción de vainilla (*Vanilla planifolia*) en México: análisis y pronóstico. *Rev. Mex. C. Agr. 11*(1). 175-187. Doi: https://doi.org/10.29312/remexca.v11i1.2065

CONAGUA. 2019. Resúmenes mensuales de temperaturas y lluvia. Available in https://smn.conagua.gob.mx/es/climatologia/temperaturas-y-lluvias/resumenes-mensuales-de-temperaturas-y-lluvias

Undata. 2023. Vanilla. Available in http://data.un.org/Data.aspx?d=FAO&f=itemCode:692&c=2,3,4,5,6,7&s=countryName:asc,elementCode:asc,year:desc&v=1

Tsay, RS. Multivariate Time Series Analysis: With R and Financial Applications; Wiley: Canada, 2014; 27-104.

Hassani, H; Yeganegi, MR. 2020. Selecting optimal lag order in Ljung–Box test. *Phys. A: Stat. Mech. Its Appl. 541*(1). 123700. Doi: https://doi.org/10.1016/j.physa.2019.123700

Granger, WJ. 1969. Investigating Causal Relations by Econometric Models and Cross-spectral Methods. *Econ. J. Econom. Soc. 37*(3). 424-438. Doi: https://doi.org/10.2307/1912791

R Core Team. 2017. R: A language and environment for statistical computing. Available in https://www.R-project.org/

Stoffer, D, Poison, N. 2023. astsa: Applied Statistical Time Series Analysis. Available in https://CRAN.R-project.org/package=astsa

Hyndman, RJ; Khandakar, Y. 2008. Automatic time series forecasting: the forecast package for R. *J. Stat. Soft. 26*(3). 1-22. Doi: https://doi.org/10.18637/jss.v027.i03

Sax, C; Eddelbuettel, D. 2018. Seasonal Adjustment by X-13ARIMA-SEATS in R. *J. Stat. Soft. 87*(11). 1-17. Doi: https://doi.org/10.18637/jss.v087.i11

Chan, K; Ripley, B. 2022. TSA: Time Series Analysis. Available in https://CRAN.R-project.org/package=TSA

Trapletti, A; Hornik, K. 2023. tseries: Time Series Analysis and Computational Finance. Available in https://CRAN.R-project.org/package=tseries

Pfaff, B. Analysis of Integrated and Cointegrated Time Series with R, 2nd ed.; New York, United States of America, 2008, 73-90. Doi: https://doi.org/10.1007/978-0-387-75967-8

Iftikhar, T; Majeed, H; Waheed, M; Zahra, SS; Niaz, M; AL-Huqail, AA. Vanilla. In Essentials of Medicinal and Aromatic Crops; Zia-Ul-Haq, M, Abdulkreem-AL-Huqail, A, Riaz, M, Farooq-Gohar, U., Eds.; Springer: Switzerland, 2023; 341-372. Doi: https://doi.org/10.1007/978-3-031-35403-8_14

Gobierno de México. 2023. Vainilla mexicana, con amplio potencial productivo y preferencia en los mercados internacionales: Agricultura. Available in https://www.gob.mx/agricultura/prensa/vainilla-mexicana-con-amplio-potencial-productivo-y-preferencia-en-los-mercados-internacionales-agricultura?idiom=es#:~:text=De%202017%20a%202021%2C%20México,superior%20a%20las%20417%20toneladas.

Borbolla-Pérez, V; Iglesias-Andreu, LG; Luna-Rodríguez, M; Octavio-Aguilar, P. 2017. Perceptions regarding the challenges and constraints faced by smallholder farmers of vanilla in Mexico. *Env. Dev. Sust*. 19. 2421-2441. Doi: https://doi.org/10.1007/s10668-016-9863-y

Ellestad, P; Pérez-Farrera, MA; Forest, F; Buerki, S. 2022. Uncovering haplotype diversity in cultivated mexican vanilla. *Am. J. Bot. 109*(7). 1120-1138. Doi: https://doi.org/10.1002/ajb2.16024

Ranadive, AS. Quality control of Vanilla beans and extracts. In Handbook of Vanilla Science and Technology, 2nd ed.; Havkin-Frenkel, D, Belanger, F, Eds.; Wiley: New York, United States of America, 2018; 239-260. Doi: https://doi.org/10.1002/9781119377320.ch15

Suárez-Barrios, R. 2015. El agroecosistema vainilla de dos regiones de México, un estudio histórico comparativo siglo XX. Master's Thesis. Colegio de Postgraduados, Fabio Altamirano, Veracruz, México. 06 de junio de 2015.

Vera-Cortés, G. 2007. Vulnerabilidad social y desastres en el Totonacapan una historia presente. Doctoral thesis. Universidad Autónoma Metropolitana, Iztapalapa, Ciudad de México, México. Junio de 2007.

Luo, H; Xu, H; Chu, C; He, F; Fang, S. 2020. High temperature can change root system architecture and intensify root interactions of plant seedlings. *Fr. plant Sci*. 11. 160. Doi: https://doi.org/10.3389/fpls.2020.00160

Reddy, KR; Brand, D; Wijerwardana, C; Gao, W. 2017. Temperature effects on cotton seedling emergence, growth, and development. *Agr. J*. 109. 1379-1387. Doi: https://doi.org/10.2134/agronj2016.07.0439

Bayman, P. The history of Vanilla in Puerto Rico: diversity, rise, fall, and future prospects. In Handbook of Vanilla Science and Technology, 2nd ed.; Havkin-Frenkel, D, Belanger, F, Eds.; Wiley: New York, United States of America, 2018; 111-120. Doi: https://doi.org/10.1002/9781119377320.ch7

Pinaria, AG; Liew, ECY; Burgess, LW. 2010. *Fusarium* species associated with vanilla stem rot in Indonesia. *Aust. Plant. Path*. 39. 176-183. Doi: https://doi.org/10.1071/AP09079

Flanagan, NS; Chavarriaga, P; Mosquera-Espinosa, AT. Conservation and sustainable use of vanilla crop wild relatives in Colombia. In Handbook of Vanilla Science and Technology, 2nd ed.; Havkin-Frenkel, D, Belanger, F, Eds.; Wiley: New York, United States of America, 2018; 85-110. Doi: https://doi.org/10.1002/9781119377320.ch6

Jackson, LE; Pascual, U; Hodgkin, T. 2007. Utilizing and conserving agrobiodiversity in agricultural landscapes. *Agr. Ecos. and Env*. 121. 196-210. Doi: https://doi.org/10.1016/j.agee.2006.12.017

Maruenda, H; del Lujan-Vico, M; Householder, JE; Janovec, JP; Cañari, C; Naka, A; Gonzalez, AE. 2013. Exploration of *Vanilla pompona* from the Peruvian amazon as a potential source of vanilla essence: quantification of phenolics by HPLC-DAD. *F. Chem*. 138. 161-167. Doi: https://doi.org/10.1016/j.foodchem.2012.10.037

Watteyn, C; Reubens, B; Azofeita-Bolaños, JB; Solano-Campos, F; Pérez-Silva, A; Karremans, AP; Muys, B. 2023. Cultivation potential of Vanilla crop wild relatives in two contrasting land use systems. *Eu. J. Agr.* 149. 126890. Doi: https://doi.org/10.1016/j.eja.2023.126890

Menchaca-García, RA; Ramos-Prado, JM; Moreno-Martínez, D; Luna-Rodríguez, M; Mata-Rosas, M; Vázquez, LM; Lozano-Rodríguez, MA. 2011. *In vitro* germination of *Vanilla planifolia* and *V. pompona* hybrids. *Rev. Col. Biotec. 13*(1). 80-84.

Yeh, C; Chen, K; Lee, Y. 2021. Asymbiotic germination of *Vanilla planifolia* in relation to the timing of seed collection and seed pretreatments. *Bot. Stu*. 62. 6. Doi: https://doi.org/10.1186/s40529-021-00311-y

Barreda-Castillo, JM; Monribot-Villanueva, JL; Velázquez-Rosas, N; Bayman, P; Guerrero-Analco, JA; Menchcaca-García, RA. 2023b. Morphological and physio-chemical responses to PEG-induced water stress in *Vanilla planifolia* and *V. pompona* hybrids. *IJMS*. 24. 4690. Doi: https://doi.org/10.3390/ijms24054690

Menchaca-García, RA. In vitro propagation of Vanilla. In Handbook of Vanilla Science and Technology, 2nd ed.; Havkin-Frenkel, D, Belanger, F, Eds.; Wiley: New York, United States of America, 2018; 181-190. Doi: https://doi.org/10.1002/9781119377320.ch12

Estudillo-Hernández, M; Rodríguez-Cabrera, R; Velázquez-García, PE; López-Castro, D; Elorza-Martínez, P. 2019. Establecimiento de *Vanilla planifolia* Andrews en acahual intercalado con *Glicidia sepium* y *Citrus cinensis*. *Rev. Biol. Agr. Tux. 7*(1). 13-18. Doi: https://doi.org/10.47808/revistabioagro.v7i1.135

Ramos-Prado, JM; Romero-Hernández, E; Sánchez-Morales, P; Jiménez-García, D; Hipólito-Romero, E. 2023. biocultural and socioeconomic dimensions of sustainability in agroforestry systems diversified with cacao and vanilla. *Rev. Mex. C. Agr. 14*(3). 401-412. Doi: https://doi.org/10.29312/remexca.v14i3.3093

Soto-Arenas, MA; Dressler, RL. 2009. A revision of the mexican and central american species of *Vanilla plumier* ex miller with a characterization of their its region of the nuclear ribosomal DNA. *Lankesteriana. 9*(3). 285-354. Doi: https://doi.org/10.15517/lank.v0i0.12065

van Noort, F. Vanilla in Dutch greenhouses: a discovery-from research to production. In Handbook of Vanilla Science and Technology, 2nd ed.; Havkin-Frenkel, D, Belanger, F, Eds.; Wiley: New York, United States of America, 2018; 157-164. Doi: https://doi.org/10.1002/9781119377320.ch10

Menchaca-García, RA; Moreno-Martínez, D; Barreda-Castillo, JM; Pérez-Silva, A; Lozano-Rodríguez, MA. 2019. Vainilla, café y cítricos: triángulo amoroso. *C. y el H*. 32. 28-33.

Chambers, AH. Vanilla (Vanilla spp.) Breeding. In Advances in plant breeding strategies: industrial and food crops, Al-Khayri, JM, Jain, SM, Johnson, DV, Eds.; Springer: Switzerland, 2019, 707-734. Doi: https://doi.org/10.1007/978-3-030-23265-8_18

Colegio de Postgraduados

Control of *Macrophomina pseudophaseolina* Crous, Sarr & Ndiaye with *Trichoderma* spp., and botanical and chemical pesticides

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ABSTRACT

Objective: The objective of the research was to evaluate in *in vitro*, greenhouse and field conditions, the effectiveness of biological, botanical and chemical pesticides for the control of *Macrophomina pseudophaseolina*.
Design/methodology/approach: *In vitro*, greenhouse and field experiments were carried out to evaluate the control effect of different pesticides for the control of *M. pseudophaseolina*.
Results: It was determined that all the evaluated strains of *Trichoderma* spp. they had a fungistatic effect against *M. pseudophaseolina*, and *T. reesei* showed the greatest antagonism and antibiosis against *M. pseudophaseolina*. High, medium and low doses of NeemAcar® and high and medium doses of Regalia® Maxx inhibited 100% the growth of *M. pseudophaseolina* mycelium. In the greenhouse, the lowest percentage of severity was obtained in the treatment with Regalia® Maxx + *T. reesei*. In the field, the lowest severity was determined with the application of NeemAcar® CE + Headline®.
Limitations on study/implications: Our results are essential for the management of this disease by producers.
Findings/conclusions: The implementation of the use of *Trichoderma* spp., botanical pesticides and chemical insecticides is recommended for the control of *M. pseudophaseolina*.

Keywords: Effectiveness, pesticide, chili crop, pathogen.

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INTRODUCTION

The chili pepper crop (*Capsicum* spp.) is produced in extensive surfaces of the world and it is an essential condiment in Mexican culinary art (Olguín and Rojas, 2018). Production of the crop is affected by the attack of fungi in the soil, including *Macrophomina phaseolina* (Tassi) Goid. (Botryosphaeriaceae), which provoke charcoal rot of the root and neck and cause significant economic losses in this crop and in more than 500 species of plants (Verma *et al.*, 2007; Kaur *et al.*, 2012).

The control of *M. phaseolina* and other soil fungi is based on the use of chemical fungicides, although the intensive use of pesticides causes collateral damage including contamination of ecosystems and foods, harm to human health, and the appearance of strains that are resistant to the products (Gisi and Sierotzki, 2008); therefore, integrated strategies are required.

Biological control, including the use of *Trichoderma* spp. against soil fungi, has been documented (McLean *et al.*, 2004; Martínez *et al.*, 2013; Diánez *et al.*, 2016). In addition to biological control, another sustainable alternative to the application of chemical fungicides is the use of botanical pesticides with fungitoxic activity provoked by the action of terpenes, phenols, alkaloids, tannins, flavonoids, phytoalexins, essential oils, and other secondary metabolites produced by the plants (Martínez, 2012).

Under *in vitro* conditions, there are studies of *Trichoderma* against various pathogens; for example, Michel-Aceves *et al.* (2009) reported that the native a strain Thzn-2 of the species *Trichoderma harzianum* has the potential of biocontrol by inhibition and class 2 antagonism on *Fusarium subglutinans* and *Fusarium oxysporum*. Other authors such as Ruiz-Cisneros (2018) reported that the species of *Trichoderma* that they used presented a positive effect on tomato plants (*Solanum lycopersicum*) by improving the variables of height, biomass, chlorophyll, yield and fruit quality under greenhouse conditions. Likewise, Castro-del Ángel *et al.* (2021) mentioned that *Trichoderma* spp. reduced the impact of *Fusarium verticillioides* in corn genotypes (*Zea mays*) in the state of Veracruz; and Shawki *et al.* (2020) reported the use of potassium silicate, niacin, and antagonists *T. harzianum*, *T. hamatum* and *Bacillus subtili* as alternatives to chemical control of *F. verticillioides*. The hypothesis of this study was that the treatments with the combinations of fungicides + plant extracts and plant extracts + *Trichoderma* spp. presented greater effectiveness for the management of the pathogen in the chili pepper crop. Because of this, the effectiveness of biological, botanical and chemical pesticides for the control of *M. pseudophaseolina* was evaluated under *in vitro*, greenhouse and field conditions.

MATERIALS AND METHODS

The strain of *M. pseudophaseolina* used was supplied by Colegio Superior Agropecuario from the state of Guerrero (CSAEGro-chADMF, accession KX757770.1), which was isolated from plant roots collected in *Capsicum annuum* L. (Solanaceae) chili pepper crop. On the other hand, the following native strains were used: 1) *Trichoderma asperellum* strain CSAEGro-Tas1-(KP639195.1), 2) *T. asperellum* strain CSAEGro-Tas2-(KP639196.1), and 3) *T. asperellum* strain CSAEGro-Tas3. In addition, the commercial strains: 4) *T. virens* strain G-41 (TvG-41) (PHC-Rootmate); 5) *Trichoderma* sp., strain TspF (Fithan®), and 6) *T. reesei* strain TrB (Bactiva®); which were obtained from the mycological collection of the Phytopathology Laboratory of the CEP-CSAEGro (Figure 1).

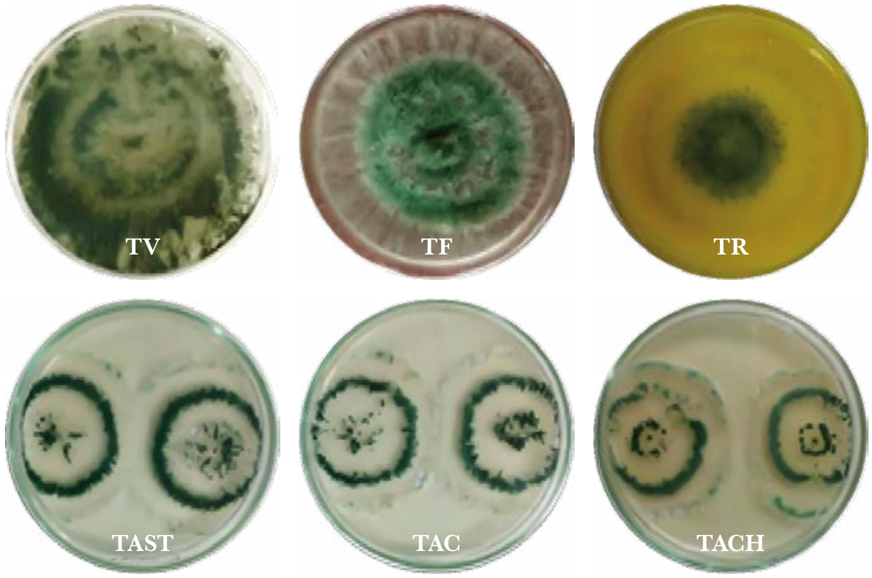

Figure 1. *Trichoderma* strains under study. TV=*T. virens* (PHC-ROOTMATE), TF=*Trichoderma* sp. (FITHAN), TR=*T. reesei* (BAC-TIVA), TAST=*T. asperellum* (native strain Santa Teresa, Guerrero), TAC=*T. asperellum* (native strain Cocula, Guerrero), TACH=*T. asperellum* (native strain from Chilapa, Guerrero).

In vitro biological control of *M. pseudophaseolina*

For this variable, two bioassays were conducted, the first was with the dual culture technique, where the treatments were: 1) Control, 2) Tas1, 3) Tas2, 4) Tas3, 5) G-41, 6) TspF and 7) TrB; first, 20 mL of PDA were emptied into the Petri dishes, then they were allowed to solidify, and a three day old 5 mm disc of *Trichoderma* spp. was placed on the corner of the dish, and in contrast a disc of *M. pseudophaseolina* was placed, giving a total of five replicas per treatment which were incubated at room temperature (±28 °C) in the laboratory; data of the variables were taken every 24 h (Larralde *et al.*, 2008).

The second bioassay was carried out with the cellophane membrane technique (Patil *et al.*, 2014), cutting discs or circles of 8.5 cm of diameter of sweet cellophane paper (of the same diameter as the Petri dish), which were wrapped (without folding) in recycled paper and placed in a poly paper bag and sterilized in the autoclave at 15 lbf in^{2-1} for one hour. Later, a circle was placed on the PDA surface in the Petri dish and a 5 mm disc of PDA + *Trichoderma* (5 days of age) was sown in the center of cellophane paper, incubated for 48 h, and then the cellophane paper was removed together with the *Trichoderma* spp. colony, leaving the secondary metabolites produced by the antagonist fungus spread on the PDA; this, with the purpose of testing its effect on the pathogenic fungus, giving a total of five replicas per treatment.

In vitro effectiveness of botanical and chemical pesticides against *M. pseudophaseolina*

Botanical (Table 1) and chemical (Table 2) pesticides were used against *M. pseudophaseolina* with the infected culture medium technique (Kumar and Mane, 2017), in a completely random experimental design with five repetitions. The experimental unit was the Petri dish with 20 mL of PDA + botanical or chemical pesticide, according to treatment (Tables 1 and 2). A 6-day-old disc (Ø=5 mm) with *M. pseudophaseolina* was sown in the center of the dish; it was incubated at 28±2 °C in light/dark and the diameter of the colony of *M. pseudophaseolina* was measured every 24 h for 72 h.

Variables evaluated

In bioassay one, the percentage of inhibition was measured every 24 h for 72 h with the Barari and Foroutan (2016) equation, where the inhibition percentage=[(a−b)/a]×100 (a=mycelium growth of the pathogen and b=mycelium growth of the pathogen in the presence of *Trichoderma* spp.).

In bioassay two, the diameter of the *M. pseudophaseolina* colony was measured every 24 h for 72 h and the percentage of inhibition of mycelium growth of the pathogen

Table 1. Doses of plant extracts evaluated *in vitro* against *M. pseudophaseolina*.

Product	Doses used in 20 mL of PDA (mg)	
[1]Neemix	High	14
	Mean	10
	Low	5
[2]Progranic® NeemAcar® CE	High	154 of *A. indica* + 42 of *C. zeylanicum*
	Mean	103 of *A. indica* + 28 of *C. zeylanicum*
	Low	51 of *A. indica* + 14 of *C. zeylanicum*
[3]Liquid Allium®	High	269
	Mean	179
	Low	90
[4]Capsi Oil	High	90 of *Cinnamomum* spp. + 60 of *P. nigrum*
	Mean	60 of *Cinnamomum* spp. + 40 of *P. nigrum*
	Low	30 of *Cinnamomum* spp. + 20 of *P. nigrum*
[5]Lipp Oil	High	120 of *L. graveolens* y *L. beriandieri* + 120 of *C. cassia* y *C. zeylanicum*
	Mean	80 of *L. graveolens* y *L. beriandieri* + 80 of *C. cassia* y *C. zeylanicum*
	Low	40 of *L. graveolens* y *L. beriandieri* + 40 of *C. cassia* y *C. zeylanicum*
[6]Cinn Oil	High	150 of *C. cassia* y *C. zeylanicum* + 45 of *L. graveolens* y *L. beriandieri* + 45 de *Allium* spp.
	Mean	100 of *C. cassia* y *C. zeylanicum* + 30 of *L. graveolens* y *L. beriandieri* + 20 de *Allium* spp.
	Low	50 of *C. cassia* y *C. zeylanicum* + 15 of *L. graveolens* y *L. beriandieri* + 10 of *Allium* spp.
[7]Alli Oil	High	270
	Mean	180
	Low	90
[8]Asphix®90	High	243.6
	Mean	162.4
	Low	81.2
[9]Regalia® (*Reynoutria sachalinensis*)	High	69
	Mean	46
	Low	23

Table 2. Doses of chemical fungicides evaluated *in vitro* against *M. pseudophaseolina*.

N°	Product*	Doses**	Doses per ha^{-1} (kg)	Doses (i.a.) used in 20 mL of PDA (mg)
1	Cercobin®-M (tiofanato de metilo)	High	0.75	10.43
2		Mean	0.62	8.61
3		Low	0.5	6.86
4	Rovral® 50 PH (iprodiona)	High	2.0	20
5		Mean	1.33	13.5
6		Low	1.0	10
7	Swich 62.5 WG (cyprodinil+fludioxonil)	High	1.2	9 of cyprodinil + 6 of fludioxonil
8		Mean	1.050	7.9 of cyprodinil + 5.3 of fludioxonil
9		Low	0.9 kg	6.8 of cyprodinil + 4.5 of fludioxonil
10	Pentaclor 600F (Quintozeno)	High	15 L	180
11		Mean	13.5 L	162
12		Low	12.0 L	144
13	Headline® (Piraclostrobin)	High	3.0 L	15
14		Mean	2.0 L	10
15		Low	1.0 L	5
16	Promyl® (Benomilo)	High	0.5 kg	5
17		Mean	0.45 kg	4.5
18		Low	0.4 kg	4
19	Sportak 45 CE (procloraz)	High	1.5 L	13.5
20		Mean	1.25 L	11.25
21		Low	1.0 L	9
22	Control	-	-	-

* The dose was calculated considering a consumption of 1,000 L of water ha^{-1}.
** The mean dose is that recommended by the product manufacturer (DEAQ, 2015).

colony was calculated with the equation by Patil *et al.* (2014), where the percentage of inhibition$=[(D1-D2)/D1]\times100$ (D1=diameter of the fungus colony growing in dishes with PDA (control), D2=diameter of the fungus colony of the pathogen growing on PDA.

Control of *M. pseudophaseolina* in greenhouse

Only seven outstanding treatments were evaluated in the greenhouse and the field, which were distributed into divided plots, with five repetitions. The experimental unit was a black polyethylene pot of 13×18 cm with 1 kg of sterilized substrate (sand+mountain soil+vermicompost 1:1:0.25 v/v) and a Creole chili pepper plant (*Capsicum annuum*). The inoculum of *M. pseudophaseolina* was multiplied in sterilized seed of *Sorghum bicolor* L. (Poaceae). Likewise, inoculum from the strains of *Trichoderma* spp. was reproduced with ground corn. Ten days after the transplant, 13 g of infested sorghum seeds were inoculated with *M. pseudophaseolina* with 4.16×10^8 spores per mL in plants with 35 d of age and then the treatments were inoculated, where *Trichoderma* spp. at 2.3×10^8 spores per mL and the different botanical and chemical pesticides were used: a) Control, b) Regalia® Maxx (0.56 mL $planta^{-1}$)+Swich® 62.5 (0.1 g), c) Regalia® Maxx (0.56 mL)+Headline® CE (0.11

mL), d) Regalia® Maxx (0.2 mL)+*T. reesei*, e) NeemAcar® CE (0.56 mL)+Swich® 62.5 (0.1 g), f) NeemAcar® CE (0.56 mL)+Headline® CE (0.11 mL), g) NeemAcar® CE (0.56 mL)+Cercobin®-M. (0.05 g).

Control of *M. pseudophaseolina* in the field

The treatments that were used in greenhouse conditions were evaluated in the field, which were distributed in divided plots. The experimental unit was three furrows, each with 5 m length and at 1.6 m of separation, with four plants of Creole chili pepper plants, at 0.7 m of distance between these (13.44 m^2). Plant transplanting was carried out at 24 d of age; 11 g of infected sorghum seeds were inoculated with *M. pseudophaseolina* at 4.5×10^5 spores per mL^{-1} in the plant neck of 79 days of age (55 days after transplanting); likewise, the application of the treatments was made with water expenditure of 112 mL per plant.

Evaluation of variables in greenhouse and field conditions

The severity of the disease was measured by using the ordinal scale by Vakalounakis and Fragkiadakis (1999) (0=healthy plant, 1=chlorotic plant, 2=withered plant and 3=dead plant; the data were transformed into percentages of severity); and the data obtained were transformed with the Van der Plank (1965) formula, where $S=\left[\sum_i/N\left(VM\right)\right]\times100$ (S=percentage of severity, $\sum_i$= sum of values observed, N=number of sick plants sampled, VM=maximum value of the scale.

Statistical analysis

The data obtained *in vitro*, in the greenhouse and in the field were analyzed separately through ANOVA and Tukey's multiple range test (α=0.05) (Steel and Torrie, 1998) with the software SAS 9.4.

RESULTS AND DISCUSSION

Biological *in vitro* control of *M. pseudophaseolina*

On average, the strains Tas1, Tas2 and TrB took 2 d to make contact with the *M. pseudophaseolina* hyphae; and TvG-41, Tas3 and TspF, 2.2, 2.4 and 2.4 days, respectively. In the competition over space and nutrients, it was determined that the strains TrB and TspF inhibited the growth of the *M. pseudophaseolina* mycelium (Figure 2A) by 61.75 and 55.5%. These values are close to 54.6% produced from the effect of *T. harzianum* against *M. phaseolina* (Singh *et al.*, 2008), and different from the values of 75.55, 72.22, 68.88 and 48.88% of inhibition, caused by *T. harzianum*, *T. reesei*, *T. hamatum* and *T. pseudokoningii*, respectively (Karthikeyan *et al.*, 2015). Likewise, it was determined that the extra-cellular metabolites of all the strains of *Trichoderma* spp. had statistically similar fungistatic action against *M. pseudophaseolina* (Figure 2B).

It is considered that the less time that is required by the strains of *Trichoderma* spp. to make contact with the pathogen, the antagonist will have better chances to compete for space, nutrition primarily by microparasitism, and the production of secondary metabolites

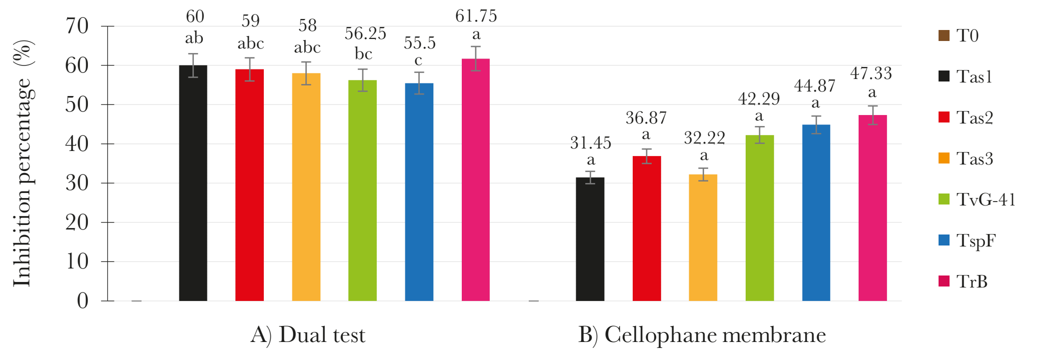

Figure 2. Percentage of inhibition of *M. pseudophaseolina* colonies at 72 h in the dual cultures and cellophane membrane bioassay. T0=Control, Tas1=*T. asperellum*, Tas2=*T. asperellum*, Tas3=*T. asperellum* native to Santa Teresa, Guerrero, Mexico, TvG-41=*T. virens* strain G-41 (PHC®-ROOTMATE®), TspF=*Trichoderma* sp. (FITHANMR), and TrB=*T. reesei* (BACTIVAMR). Letters that are equal are not statistically significant (Tukey α=0.05).

and high production of degrading enzymes such as chitinase, protease and lignase, which degrade the cell wall of the phytopathogenic fungus and use the cytoplasmic content for the nutrition of *Trichoderma* (De Marco *et al.*, 2004; Harman, 2006; Gonzalez *et al.*, 2012; Hernández-Melchor *et al.*, 2019).

The products Progranic® NeemAcar CE (low, medium and high doses) and Regalia® Maxx (high and medium doses) obtained the highest effectiveness, because they exert fungicide action on the causal agent of charcoal rot, with 100% of pathogen inhibition (Table 3).

Effect of botanical and chemical pesticides on *M. pseudophaseolina*

It was found that the treatments with organic and chemical products caused highly significant differences in the percentage of inhibition of the *M. pseudophaseolina* colonies ($P<0.0001$) (Figure 3). A fungicide effect against *M. pseudophaseolina* was obtained with the applications of high and medium doses of Regalia® and with all the doses of NeemAcar® CE. All the other treatments presented fungistatic effect.

The low dose of *R. sachalinensis* decreased the growth of *M. pseudophaseolina* by 87.98%. The effectiveness of the Progranic, Alli Oil, Capsi-Oil, Cinn Oil, and Asphix® extracts decreased when reducing the doses of the products; however, it was found that the low dose of Lipp-Oil was more effective than the higher dose, perhaps due to the interaction in high concentrations of all the components of this product (*L. graveolens* + *L. berlandieri* + *C. cassia* + *C. zeylanicum*), which could stimulate the mycelium growth of *M. pseudophaseolina*. When it comes to botanical pesticides, their inhibitory effect is because the secondary metabolites of the plants with which they are manufactured, such as phenols, terpenoids, alkaloids, carboxyl acids, and fatty acids, show insecticide properties (Avalos and Perez, 2009).

In this regard, Tandel *et al.* (2010) mentioned that with the extract of *A. cepa*, the effectiveness was 98.14%; Javaid and Asma (2011) also reported that the extracts of *Syzygium cumini* (L.) Skeels, *Eucalyptus citriodora* Roxb., *A. indica* L. and *Melia azederach* L., reduced

Table 3. Biological effectiveness of organic extracts on the growth of *M. pseudophaseolina* at 72 h.

Product	Dose	Mean (%)
Neemix 4.5% CE	High	12.35
	Mean	8.35
	Low	0
Progranic® NeemAcar CE	High	100
	Mean	100
	Low	100
Liquid Allium®	High	30.68
	Mean	22.33
	Low	23.68
Capsioil	High	77.33
	Mean	66.68
	Low	61.35
Lippoil	High	60.33
	Mean	69.65
	Low	89.33
Cinnoil	High	51.33
	Mean	49.33
	Low	41.33
Allioil	High	18.98
	Mean	0
	Low	0
Asphix® 90	High	27
	Mean	4
	Low	0
Regalia Maxx®	High	100
	Mean	100
	Low	87.98
Control	--	--

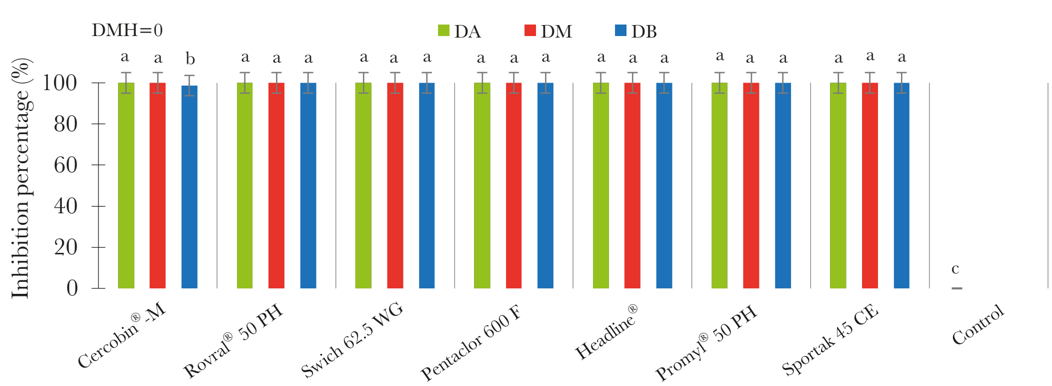

Figure 3. Percentage of inhibition of *M. pseudophaseolina* colonies due to the effect of chemical fungicides. Columns with the same literal are not statistically different. DMH=Tukey's minimum honest difference, α=0.05. DA=High dose, DM=Medium dose and DB=Low dose.

the biomass of *M. phaseolina*, while the extracts in acetate and chloroform of *A. indica* inhibited, the growth of *M. phaseolina* by 81 and 90%, respectively.

Muzammil *et al.* (2014) and Meena *et al.* (2014) reported that the extract of *A. sativum* reduced by 100% the growth of *M. phaseolina* in sunflower. Savaliya *et al.* (2015) also found that the extracts of *A. sativum*, *A. cepa* and *Zingiber officinale* Rosc., inhibited the growth of *M. phaseolina* by 77.65, 63.98 and 32.34%, respectively. In addition, they reported that the extracts of *A. sativum* and *A. cepa* suppressed the formation of microsclerotia of this phytopathogen. Baldiga *et al.* (2013) reported that carbendazim, iprodione and penflufen + fluoxastrobin applied at 40 mg L^{-1} had effectiveness of 5.6, 7.2 and 12.5%, respectively, against *M. phaseolina*. Reznikov *et al.* (2016) reported that pyraclostrobin + thiophanate-methyl increased the percentage of root development of soy seeds infected with *M. phaseolina*.

Control of *M. pseudophaseolina* in greenhouse conditions

Significant differences were found between treatments only in samples two and three. From the first to the last evaluation, it was found that the Regalia® Maxx + Swich 62.5 WG treatment stood out, because it presented the lowest averages of severity of charcoal rot. In evaluation three it was determined that in the plants treated with the mixture of these two products, there was 94.44% less severity than in the treatments with NeemAcar® + Cercobin®-M and Regalia® Maxx. Likewise, there was no difference in the application method, although the severity was slightly lower in the treatments applied preventively; that is, the time of application did not influence the severity of the disease in the chili pepper plants (Table 4).

Table 4. Comparison of the severity of *Macrophomina pseudophaseolina* on three evaluation dates under greenhouse conditions.

Treatments	Days after inoculation		
	7	14	21
T0	44.45a†	94.45a	100a
N1	5.56a	5.56b	5.56bc
N2	27.78a	33.33ab	27.78bc
N3	27.78a	5.56b	0c
N4	16.67a	50ab	50abc
N5	16.67a	66.67ab	66.67ab
N6	27.78a	72.22ab	100a
DMH	48.154	69.313	64.205
Valor of P	0.2758	0.0020	$<$0.0001

DT0=Control, N1=Regalia® Maxx + Swich 62.5 WG, N2=Regalia® Maxx + Headline, N3=Regalia® Maxx + *T. reesei*, N4=NeemAcar CE + Swich 62.5 WG, N5=Neemacar CE + Headline CE, N6=NeemAcar CE + Cercobin®-M. † Values with the same letters in the same column are not statistically different. DMH: Tukey's minimum honest difference α=0.05.

The effect of the application of Regalia® Maxx may be because the components of this extract can stimulate the synthesis of phytoalexins and C-glycosdsil (cucumarin) which accumulate in the penetration site of the fungus, in addition to promoting the lignification of the cell wall and an increase in the activity of the enzymes chitinases, peroxydases and β-1,3 glucanasas, which affect the colonization and survival of the pathogen (Fofana *et al.*, 2005) Similarly, Margaritopoulou *et al.* (2020) reported that the extract of *Reynoutria sachalinensis* causes defenses in the plants, especially as consequence of the induction of the salicylic acid path. Su *et al.* (2012) mention that the extract of *R. sachalinensis* can induce resistance in the plants through induction of phytoalexins and production of phenolic compounds, an increase in the production of proteins related to the defense, and accumulation of species that are reactive to oxygen, lignification, and formation of papillas on the cell walls.

The favorable effect obtained with the *Trichoderma* strains is because this bio controlling fungus presents fast growth, broad ecological plasticity (Harman, 2006), and mechanisms of direct action such as competition over the substrate, antibiosis, and microparasitism against *M. phaseolina* and other phytopathogens that live in the soil (Khaledi and Taheri, 2016). In addition to these effects, *Trichoderma* spp. colonize the root zone limiting their growth to the external layers of the root and do not penetrate the vascular bundle, which improves the development of the plants (Poveda, 2020).

Integrated control of *M. pseudophaseolina* in the field

In the statistical analysis, significant differences were detected only in samples two and three. It was found that the treatments with NeemAcar® CE + Headline CE and NeemAcar® + Cercobin®-M presented the lowest averages (32.9 and 33.3%) of severity, respectively, making them the most effective treatments to counteract charcoal rot (Table 5).

NeemAcar® CE is formulated from *A. indica* and *C. zeylanicum* and presently it is known that the first has several bioactive compounds (Atawodi and Atawodi, 2009); meanwhile, Lu *et al.* (2011) mention that cinnamon oil (*C. zeylanicum*) shows synergistic effects when

Table 5. Comparison of the percentage of severity of *Macrophomina pseudophaseolina* on the three evaluation dates.

Treatments	Days after inoculation		
	7	14	21
Control	42.1 a*	63.4 a	84.7 a
Regalia® Maxx + Swich 62.5 WG	40.7 a	41.2 ab	37.5 b
Regalia® Maxx + Headline CE	29.0 a	44.0 ab	40.7 b
Regalia® Maxx + *T. reesei*	31.5 a	25.9 b	39.8 b
NeemAcar® CE + Swich 62.5 WG	43.5 a	39.8 ab	49.5 b
NeemAcar® CE + Headline CE	33.8 a	33.3 ab	32.9 b
NeemAcar® + Cercobin®-M	21.2 a	25.9 b	33.3 b

*Values with the same letters in the same column are not statistically different (Tukey, α=0.05).

it is combined with other oils of plant origin to control both "gram positive" and "gram negative" bacteria, which explains why the use of NeemAcar® was superior.

The indiscriminate use of pesticides has reduced and damaged the agricultural activity of the country (Zepeda-Jazo, 2018), not just in chili pepper cultivation, but in every crop. Ortiz *et al.* (2014) suggest that despite the regulations and restrictions of pesticide use, they can represent a serious problem not only in the soils and waters in Mexico, but also for the health of workers and populations exposed. The appearance of resistance or multiple resistances from pests, diseases and weeds to different active ingredients must be mentioned. This study's results will be very important for the control of this disease by producers of chili pepper, because it includes strategies of integrated management, not only in the use of chemical control; however, some of the implications for the future are to take into account the cost/benefit and to compare these management strategies with conventional management, as well as the elaboration of these types of extracts to try to reduce the contamination from chemical pesticides and the production costs of this crop for producers.

CONCLUSIONS

The research determined that all the strains evaluated of *Trichoderma* spp. had a fungistatic effect against *M. pseudophaseolina*. The high, medium and low dose of NeemAcar® and high and medium dose of Regalia® Maxx inhibited the growth of the mycelium of *M. pseudophaseolina* by 100%. The lowest percentage of severity was obtained under greenhouse conditions, in the treatment with Regalia® Maxx + *T. reesei*. The lowest severity was determined in the field with the application of NeemAcar® CE + Headline®.

REFERENCES

Atawodi, S.E., Atawodi, J.C. (2009). *Azadirachta indica* (neem): a plant of multiple biological and pharmacological activities. *Phytochemistry Reviews*, 8, 601–620. https://doi.org/10.1007/s11101-009-9144-6

Avalos, G.A, Pérez, U.C.E. (2009). Metabolitos secundarios de plantas. *Reduca (Biología) Serie Fisiología Vegetal, 2*(3),119-1445.

Baldiga, T.R.F., Avozani, A., Durante, D.L.A., Melo, R.E., Zoldan, S.M., Garcés, F.F.R. (2013). *In vitro* mycelial sensitivity of *Macrophomina phaseolina* to fungicides. *Pesquisa Agropecuária Tropical, 43*(4), 460-466. https://doi.org/10.1590/S1983-40632013000400014

Barari, H., Foroutan, A. (2016). Biocontrol of soybean charcoal root rot disease by using *Trichoderma* spp. *Cercetari Agronomice in Moldova, 49*(2), 41-51. https://doi.org/10.1515/cerce-2016-0014

Castro-del Ángel, E., Sánchez-Arizpe, A., Galindo-Cepeda, Ma.E., Vázquez-Badillo, E. (2021). Efecto de especies de *Trichoderma* sobre la incidencia de *F. verticillioides* en cuatro genotipos de maíz. *Tropical and Subtropical Agroecosystems,* 24, 67.

De Marco, J.L., Valadares, I.M.C., Felix, C.R. (2004). Purification and characterization of an N-acetylglucosaminidase produced by a *Trichoderma harzianum* strain which controls *Crinipellis perniciosa*. *Applied Microbiology Biotechnology, 64*(1), 70–75. https://doi.org/10.1007/s00253-003-1490-5

DEAQ. 2015. Diccionario de Especialidades Agroquímicas y Fertilizantes. Edición 25. PLM®. México.

Diánez, M.F., Santos, M., Carretero, F., Marín, F. (2016). *Trichoderma saturnisporum*, a new biological control agent. *Journal of the Science of Food and Agriculture, 96*(6), 1934-1944. https://doi.org/10.1002/jsfa.7301

Fofana, B., Benhamou, N., Mcnally, D., Labbé, C., Séguin, A., Bélanger, R. (2005). Suppression of induced resistance in cucumber through disruption of the flavonoid pathway. *Phytopathology, 95*(1), 114-123. https://doi.org/10.1094/PHYTO-95-0114

Gisi, U., Sierotzki, H. (2008). Fungicide modes of action and resistance in downy mildews. *European Journal of Plant Pathology, 122*(1), 157-167. https://doi.org/10.1007/s10658-008-9290-5

González, I., Infante, D., Martínez, B., Arias, Y., González, N., Miranda, I., Peteira B. (2012). Inducción de quitinasas y glucanasas en cepas de *Trichoderma* spp. promisorias como agentes para el control biológico. *Biotecnologia Aplicada, 9*(1), 12-16

Harman, G.E. (2006). Overview of mechanisms and uses of *Trichoderma* spp. *Phytopathology, 96*(1), 190-194. https://doi.org/10.1094/PHYTO-96-0190

Hernández-Melchor, D.J., Ferrera-Cerrato, R., Alejandro, A. (2019). *Trichoderma*: Importancia agrícola, biotecnológica, y sistemas de fermentación para producir biomasa y enzimas de interés industrial. *Chilean Journal of Agricultural and Animal Sciences, 35*(1), 98-112. https://doi.org/10.4067/S0719-38902019005000205

Javaid, A., Asma, R.H. (2011). Antifungal activity of leaf extracts of some medicinal trees against *Macrophomina phaseolina*. *Medicinal Plants Research, 5*(13), 2868- 2872.

Karthikeyan, V., Brindha, S., Annadurai, B., Gangwar, S.K. (2015). Biological control of *Macrophomina phaseolina* (Tassi) Goid. root rot in *Vigna mungo* (black gram) with *Trichoderma* spp. *International Journal of Advanced Biological Research, 5*(2), 118- 127.

Kaur, S., Singh, G., Kaur, S., Vallad, G.E., Chand, S., Bahadur, V. (2012). Emerging phytopathogen *Macrophomina phaseolina*: biology, economic importance and current diagnostic trends. *Critical Reviews in Microbiology, 38*(2), 136-151.

Khaledi, N., Taheri, P. (2016). Biocontrol mechanisms of *Trichoderma harzianum* against soybean charcoal rot caused by *Macrophomina phaseolina*. *Journal of Plant Protection Research, 56*(1), 21-31. https://doi.org/10.1515/jppr-2016-0004

Kumar, P., Mane, S.S. (2017). Studies on the Compatibility of Biocontrol Agents with Certain Fungicides. *Journal of Pharmacognosy and Phytochemistry, 6*(3), 1639-1644.

Larralde, C.C., Santiago, M., Sifuentes, A., Rodríguez, I., Shirai, K., Narváez, J. (2008). Biocontrol potential and polyphasic characterization of novel native *Trichoderma* strains against *Macrophomina phaseolina* isolated from Sorghum and common bean. *Applied Microbiology and Biotechnology, 80*(1), 167-177. https://doi.org/10.1007/s00253-008-1532-0

Lu, F., Ding, Y.C., Ye, X.Q., Ding, Y.T. (2011). Antibacterial effect of cinnamon oil combined with thyme or clove oil. *Agricultural Sciences in China, 10*(9), 1482–1487. https://doi.org/10.1016/S1671-2927(11)60142-9

Margaritopoulou, T., Toufexi, E., Kizis, D., Balayiannis G., Anagnostopoulos C., Theocharis A., Rempelos L., Troyanos Y., Leifert C., Markellou, E. (2020). *Reynoutria sachalinensis* extract elicits SA-dependent defense responses in courgette genotypes against powdery mildew caused by *Podosphaera xanthii*, scientific reports, 10, 3354. https://doi.org/10.1038/s41598-020-60148-6

Martínez, B., Infante, D., Reyes, Y. (2013). *Trichoderma* spp. y su función en el control de plagas en los cultivos. *Revista de Protección Vegetal, 28*(1), 1-11.

Martínez, H.A., Laurentin, H. (2012). Caracterización fenotípica y molecular de *Macrophomina phaseolina* (Tassi) Goid. proveniente de la zona de producción de Ajonjolí en Venezuela. *Bioagro, 24*(3), 187-196.

McLean, K.L., Dodd, S.L., Sleight, B.E., Hill, R.A., Stewart, A. (2004). Comparison of the behavior of a transformed hygromycin resistant strain of *Trichoderma atroviride* with the wild-type strain. *New Zealand Plant Protection Society, 57*(1), 72-76. https://doi.org/10.30843/nzpp.2004.57.6892

Meena, P.N., Tripathi, A.N., Gotyal, B.S., Satpathy, S. (2014). Bio-efficacy of phytoextracts and oil cakes on *Macrophomina phaseolina* (Tassi) causing stem rot disease of jute, *Corchorus* spp. *Journal of Applied and Natural Science, 6*(2), 530- 533. https://doi.org/10.31018/jans.v6i2.494

Michel-Aceves, A.C., Otero-Sánchez, M.A., Solano-Pascacio, L.Y., Ariza-Flores, R., Barrios-Ayala, A., Rebolledo-Martínez, A. (2009). Biocontrol *in vitro* con *Trichoderma* spp. de *Fusarium subglutinans* (Wollenweb. y Reinking) Nelson, Toussoun y Marasas y *F. oxysporum* Schlecht., Agentes Causales de la "Escoba de Bruja" del Mango (*Mangifera indica* L.). *Revista Mexicana de Fitopatología, 27*(1), 18-26.

Muzammil, H., Muhammad, U.G., Muhammad, I.H., Muhammad, A.Z., Touseef, H., Mubashar, R., Ulhaq, A. (2014). *In vitro* evaluation of fungicides and plant extracts for suppressing mycelium growth of *Macrophomina phaseolina* causes charcoal rot of sunflower. *Journal of Applied Agricultural Sciences, 6*(2), 136-140

Olguín L.M., Rojas G.D. (2018). El chile, principal condimento de la cocina mexicana. https://unamglobal.unam.mx/el-chile-principal-condimento-de-la-cocina-mexicana/

Ortíz, I., Avila-Chávez, M. Torres, L. (2014). Plaguicidas en México: usos, riesgos y marco regulatorio. *Revista Latinoamericana de Biotecnología Ambiental y Algal*, 5. https://doi.org/10.7603/s40682-014-0003-9

Patil, N.N., Waghmo de M.S.; Gaikwad, P.S.; Gajbhiye, M.H.; Gunjal, A.B., Nawani, N., Kapadnis, B.P. (2014). Potential of *Microbispora* sp. V2 as biocontrol agent against *Sclerotium rolfsii*, the causative agent of southern blight of *Zea mays* L. (Baby corn) *in vitro* studies. *Indian journal of experimental biology*, *52*(1), 1147- 1151.

Poveda, J. (2020). *Trichoderma parareesei* favorsthe tolerance of rapeseed (*Brassica napus* L.) tosalinity and drought due to a chorismate mutase. *Agronomy, 10*(1), 118. https://doi.org/10.3390/agronomy10010118

Reznikov, S., Vellicce, G.R., González, V., De Lisi, V., Castagnaro, A.P., Ploper, L.D. (2016). Evaluation of chemical and biological seed treatments to control charcoal rot of soybean. *Journal of General Plant Pathology*, 82, 273–280. https://doi.org/10.1007/s10327-016-0669-4

Ruiz-Cisneros, M.F., Ornelas-Paz, J.J., Olivas-Orozco, G.I., Acosta-Muñiz C.H., Sepúlveda-Ahumada, D.R., Pérez-Corral, D.A., Rios-Velasco, C., Salas-Marina, M.A., Fernández-Pavía, S.P. (2018). Efecto de *Trichoderma* spp. y hongos fitopatógenos sobre el crecimiento vegetal y calidad del fruto de jitomate. *Revista Mexicana de Fitopatología, 36*(3), 444-456. https://doi.org/10.18781/R.MEX.FIT.1804-5

SAS Institute Inc. (2016). SAS/SHARE® 9.4: User's Guide, Second Edition. Cary, NC: SAS Institute Inc.

Savaliya, A.V., Bhaliya, M.C., Marviyand, B.P., Akbari, F.L. (2015). Evaluation of phytoextracts against *Macrophomina phaseolina* (Tassi) Goid. Causing root rot of sesame. *Journal Biopest, 8*(2), 116-119.

Shawki, K.F.M., Elsayed, A.B.B., Abido, W.A.E., Shabana, Y.M. (2020). Using green chemicals and biological control agents for controlling the seed-borne pathogen *Fusarium moniliforme* in sugar beet. *Journal of Plant Protection and Pathology, 11*(2), 63-72. https://doi.org/10.21608/jppp.2020.78905

Singh, V., Ranaware, M.A., Nimbkar, N. (2008). Bio efficacy of antagonists against root-rot fungus *Macrophomina phaseolina* of safflower. 7th International Safflower Conference Wagga Wagga Australia.

Steel, G.D., Torrie, J.H. (1998). Bioestadística: principios y procedimientos. Segunda Edición. Mc Graw Hill. México, D. F. 392 pp.

Su, H., Blair, R., Johnson, T.B., Marrone, P.G. (2012). Regalia® bioprotectant in plant disease management. *Outlooks on Pest Management*, 23, 30-34. https://doi.org/10.1564/23feb09

Tandel, D.H., Sabalpara, A.N., Pandya, J.R. (2010). Efficacy of Phytoextracts on *Macrophomina phaseolina* (Tassi) Goid causingLeaf Blight of Green Gram. *Agricultural and Food Sciences, Environmental Science*, 2, 1-5.

Vakalounakis, D.J., Fragkiadakis, G.A. (1999). Genetic diversity of *Fusarium oxysporum* isolates from cucumber: Differentiation by pathogenicity, vegetative compatibility, and RAPD fingerprinting. *Phytopathology,* 89, 161-168. https://doi.org/10.1094/PHYTO.1999.89.2.161

Van der Plank, J.E. (1965). Dynamics of epidemics of plant disease. *Science*, 147, 120-4.

Verma, M., Brar, S.K., Tyag, R.D., Surampalli, R.Y., Valero, J.R. (2007). Antagonistic fungi, *Trichoderma* spp.: Panoply of biological control. *Biochemical Engineering Journal*, 37,1-20. https://doi.org/10.1016/j.bej.2007.05.012

Zepeda-Jazo, I. (2018). Manejo sustentable de plagas agrícolas en México. *Agricultura, sociedad y desarrollo, 15*(1), 99-108.

AGRO PRODUCTIVIDAD

Physicochemical characterization of four varieties of prickly pear (*Opuntia* spp.)

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ABSTRACT

Objective: To characterize the physicochemical properties of the pulp from four varieties of prickly pear collected in three localities from the municipality of Salinas, San Luis Potosí.

Design/Methodology/Approach: The following characteristics were recorded in 15 fruits of each variety: fruit size (equatorial and polar diameter), fresh biomass of total fruit, peel, and locule. Nine fruits per replicate were used (one fruit equals one replicate) to analyze moisture, ash content, total soluble solids, pH, titratable acidity, and total soluble sugars.

Results: The physical differences of the fruits, total fresh weight, and equatorial and polar diameter match the typical characteristics of each variety. The largest fruits were (in descending order): Cristalina, Fafayuca, Naranjona, and Cardona. The highest moisture percentage (87.34%) was reported in Cristalina. Varieties such as Fafayuca from La Reforma and Naranjona from Salinas de Hidalgo had the highest and lowest concentration of total soluble sugars (25.84 and 11.46 g 100 g^{-1} FW, respectively). The fruits had a titratable acidity from 0.01 to 0.03% citric acid, while total soluble solids ranged from 11.60 to 15.84 °Brix.

Study Limitations/Implications: Harvesting was based on the visual parameters established by the farmers.

Findings/Conclusions: Fruits harvested from backyard orchards (<500 m^2) had fruits of greater weight, which are used for self-consumption. The amount of total soluble solids and total soluble sugars per variety was different between localities, indicating that farmers from different localities have different harvesting criteria.

Key words: sugars, chemical characteristics, backyard orchards, fruit morphology, prickly pear.

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INTRODUCTION

The Altiplano Potosino is characterized by a great diversity of prickly pear cactus species, which can be found both in wild populations and in backyard orchards. In recent decades, a great deal of attention has been paid to the benefits of the *Opuntia* genus, because all of its parts (including cladode, pulp and peel of the fruits, and seeds) have shown potential to treat several diseases (Abbas *et al.*, 2022). As a fresh product and living tissue, pricky pears are susceptible to physical, chemical, and biological modifications between harvest and consumption, affecting its quality and reducing its postharvest life, which is approximately

10-15 days (Sandoval-Trujillo *et al.*, 2019). These issues limit their long-term storage and worldwide distribution (Feugang *et al.*, 2006). Prickly pear is an oval or cylindrical fleshy berry, 5 cm to 10 cm long and 4 cm to 8 cm wide. Its total weight generally ranges from 80 to 200 g (Martins *et al.*, 2023a), which are divided into three components: peel (30-40%), pulp (60-70%), and seeds (2-10%). Fruit weight can vary greatly depending on cultivar, cladode load, and environmental conditions. They can sometimes reach a weight of up to 250 g. However, a commercial fruit should not weigh less than 120 g (Cabrera, 2021), although wild fruits have recorded less than 60 g (Reyes-Agüero *et al.*, 2009). Prickly pear consumption in the agri-food sector has increased due to its organoleptic properties, nutritional value, and health benefits (Silva *et al.*, 2021). The objective of the study was to assess the physicochemical properties of pulp in four varieties of prickly pear from three localities in the municipality of Salinas, San Luis Potosí, Mexico.

MATERIALS AND METHODS

Collection of plant material

Opuntia fruit (prickly pear) varieties such as Cardona (*Opuntia streptacantha*), Naranjona (*O. megacantha*), Cristalina, and Fafayuca (*O. albicarpa*) were harvested at consumption maturity from June to August 2022, according to the visual parameters (size and fullness of fruits) established by the producers.

The four prickly pear varieties were harvested in La Reforma, Diego Martin and Salinas de Hidalgo in the municipality of Salinas, San Luis Potosi, in self-consumption backyard orchards (<500 m^2), as well as in commercial backyard orchards (>1 ha) (Figure 1, Table 1).

Fruit size (polar and equatorial diameter): The polar diameter of the fruit (base to apex) and the equatorial diameter (middle zone) of 15 fruits of each variety were measured using a digital vernier caliper (Karlen®, Mexico) with 0.01 mm accuracy.

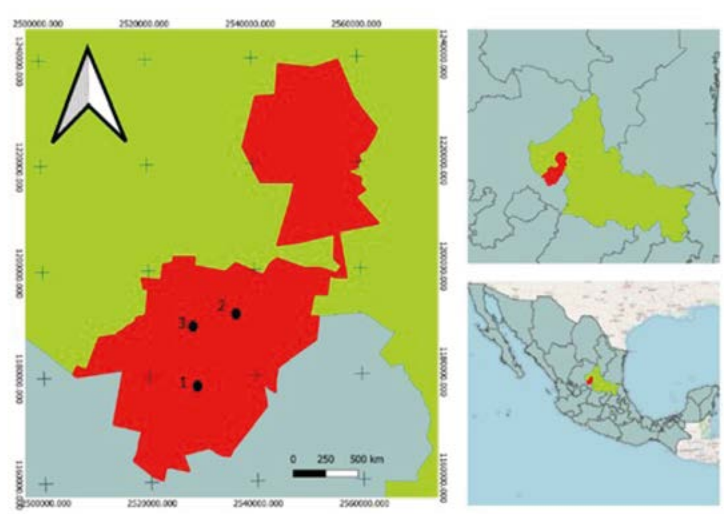

Figure 1. Geographical location of the three localities of the Altiplano Potosino, Mexico where prickly pear varieties were harvested: 1) Salinas de Hidalgo, 2) La Reforma, 3) Diego Martín. Figure developed by the authors.

Table 1. Prickly pear varieties harvested in three localities of the municipality of Salinas, San Luis Potosí, backyard area, and geographical location of harvesting sites.

Variety	Locality	Area	Longitude	Latitude	Altitude (masl)
Cardona Naranjona Cristalina Fafayuca	Salinas de Hidalgo	>1 ha	22.63078° N	101.73632° W	2082
	La Reforma	<500 m^2	22.75623° N	101.64686° W	2080
	Diego Martín	<500 m^2	22.73049° N	101.71960° W	2040

Total fresh weight: A Velab™ VE-1000 analytical electronic balance was used to determine the individual weight of the fruit, peel, and locule (pulp and seeds).

Chemical characteristics of four varieties of prickly pear

Nine fruits per variety were used to determine moisture, ash content, total soluble solids, pH, titratable acidity, and total soluble sugars.

Moisture. A 0.5 g sample of prickly pear fruit pulp was weighed with an OHAUS™ MB45 moisture analyzer to determine the moisture percentage, expressing the results as a percentage.

Ash content. The ash content was determined according to the NMX-F-066-S-1978 standard. Five g of the sample were weighted in a porcelain crucible, previously brought to constant weight for 12 h at 75 °C and slowly pre-incinerated until it stopped smoking. Subsequently, the crucible was placed in a muffle at 550 °C for 5 h to be fully burned and then allowed to cool in a desiccator to record the weight of the crucible with the ashes.

Total Soluble Solids. An Atago 2311 digital refractometer (Master Professional) was used to determine the total soluble solids of the pulp, expressed in °Brix at 20 °C.

pH. pH was determined with a PH 700 pH Meter Kit (Apera Instruments). Two grams of pulp were mixed in 20 mL of distilled water; this mixture was filtered before determining the pH.

Titratable acidity. The pH was measured to analyze the titratable acidity. Three grams of pulp were homogenized in 30 mL of distilled water. The mixture was titrated with NaOH (Macron Fine Chemicals) 0.01 N, until a pH of 8 was reached, with the help of an Orion Star™ A25 potentiometer (Thermo Scientific).

Total soluble sugars. Total soluble sugars were quantified according to the Anthrone method (Montreuil *et al*., 1997).

Statistical Analysis

The morphological and physicochemical characteristics were subjected to an analysis of variance (ANOVA) by variety between collection sites. The difference between means per sample was assessed with Tukey's test (α=0.05) using STATISTICA version 7.0 and Minitab version 18.0.

RESULTS

Total fresh weight of the fruit (TFWF). The results showed that the TFWF and polar fruit diameter (PFD) of Cardona (*O. streptacantha*) were significantly higher in La

Reforma than in Salinas de Hidalgo and Diego Martin (Table 2), indicating that fruit from orchards with an area of less than 500 m^2 in La Reforma may be more suitable for the production of Cardona fruit. Meanwhile, the TFWF of the Naranjona variety was significantly higher in La Reforma than in the other two locations assessed; however, its equatorial (EFD) and polar (PFD) diameters were the same in La Reforma and Diego Martín. For the Cristalina variety, the heaviest fruits with the highest EFD and PFD were located at Diego Martín. Finally, for the Fafayuca variety, the heaviest and biggest fruits were recorded in La Reforma. According to Patil and Dagadkhair (2019), a TFWF between 67 and 216 g per fruit (depending on the species) has been reported in the genus *Opuntia*. Meanwhile, a TFWF from 23.5 to 116.4 and 29.8 to 256.4 g has been reported in *O. streptacantha* and *O. megacantha*, respectively (Reyes-Agüero *et al.*, 2009). These values are similar to those found in this work for the same species.

Moisture content. While the Cardona variety harvested in La Reforma had a higher moisture content than the fruit from Diego Martín and a similar percentage than commercial use fruit (Salinas de Hidalgo), the moisture content of the Naranjona, Cristalina, and Fafayuca varieties did not show significant differences (Table 3) in the three studied localities. Among the species analyzed and those reported in other research works (Zenteno-Ramírez, 2016; Cabrera, 2021), the moisture percentage ranges from 80 to 90% in cultivated and wild varieties.

Ash content. The ash percentage of the Cardona variety was statistically equal in the three locations, with 0.29 to 0.33% less ash content than those recorded by Martins *et al.* (2023b). Orange varieties reported 0.35% (Paucara and Del Castillo, 2021) and 0.37% (Díaz *et al.*, 2007). These values were similar to those determined for the Naranjona variety (0.35 to 0.40%) and no significant difference was detected between locations (Table 3). Regarding

Table 2. Analysis of physical characteristics of four prickly pear varieties in three localities of the municipality of Salinas, San Luis Potosí.

Variety	Locality	TFWF (g)	PFD (cm)	EFD (cm)
Cardona	Salinas de Hidalgo	$51.94^{b}\pm1.69$	$4.71^{b}\pm0.04$	$4.23^{b}\pm0.07$
	La Reforma	$73.22^{a}\pm3.95$	$5.59^{a}\pm0.14$	$4.80^{a}\pm0.14$
	Diego Martín	$56.44^{b}\pm3.86$	$4.88^{b}\pm0.22$	$4.48^{ab}\pm0.13$
Naranjona	Salinas de Hidalgo	$70.06^{c}\pm6.14$	$6.91^{b}\pm0.17$	$4.57^{b}\pm0.16$
	La Reforma	$126.57^{b}\pm6.74$	$7.75^{a}\pm0.27$	$5.73^{a}\pm0.16$
	Diego Martín	$160.55^{a}\pm8.18$	$7.56^{ab}\pm0.25$	$6.09^{a}\pm0.12$
Cristalina	Salinas de Hidalgo	$120.59^{c}\pm7.03$	$7.29^{c}\pm0.16$	$5.41^{b}\pm0.14$
	La Reforma	$142.30^{b}\pm6.03$	$8.06^{b}\pm0.48$	$5.80^{b}\pm0.23$
	Diego Martín	$185.46^{a}\pm7.75$	$9.56^{a}\pm0.30$	$6.14^{a}\pm0.12$
Fayayuca	Salinas de Hidalgo	$124.72^{b}\pm4.53$	$7.72^{a}\pm0.22$	$5.44^{ab}\pm0.11$
	La Reforma	$148.41^{a}\pm7.38$	$7.90^{a}\pm0.26$	$5.78^{a}\pm0.12$
	Diego Martín	$86.72^{c}\pm3.01$	$7.61^{a}\pm0.14$	$4.69^{b}\pm0.05$

TFWF=total fresh weight of fruit; PFD=polar fruit diameter; EFD=equatorial fruit diameter; ±=accuracy of an approximation; S.E.=standard error. For each variable, means per variety between locations that do not share a letter are significantly different according to Tukey (α=0.05).

the varieties with green pulp (Cristalina and Fafayuca), the percentages ranged from 0.18 to 0.26%; these figures are lower than the 0.40% value reported by Díaz *et al.* (2007).

Total Soluble Solids (TSS). The results showed that the TSS of the Cardona prickly pear was similar in the three locations, with an average of 14.27 °Brix. In Salinas de Hidalgo, Naranjona prickly pear had the highest TSS content, while lower TSS contents were recorded in La Reforma and Diego Martin, with no significant differences between locations (Table 3). The Cristalina prickly pear had a significantly higher TSS content in Salinas de Hidalgo and Diego Martín than in La Reforma. Fafayuca prickly pear did not show significant differences between locations (Table 3).

Overall, the results of this study suggest that the TSS content of the prickly pear varieties assessed is adequate for fresh consumption. However, the Naranjona prickly pear from La Reforma could be a more attractive option for the market, as it statistically showed the highest TSS of the three locations. Prickly pear has a higher TSS content than other widely consumed fruits, such as peaches, apples, plums, apricots, cherries, and melons, which have values of 7.5 to 11.0 °Brix (Corrales-García, 2011).

Potential Hydrogen (pH). Table 3 shows that the pH of pulp from the prickly pear varieties assessed had a very narrow range (6.11-6.88) (Table 3), which falls within the range (5.3-6.6) reported for prickly pears by other researchers (Feugang *et al.*, 2006). This result confirms that prickly pear has lower acid level (pH>4.5) than citrus fruits (orange, lemon, etc.); however, it is more vulnerable to microbiological deterioration (Lamia *et al.*, 2018).

Titratable acidity (TA). The titratable acidity of the assessed varieties is lower than the acidity of raspberry (1.05%), strawberry (3.63%), grapefruit (1.41%), Mexican plum

Table 3. Analysis of the chemical characteristics of the pulp of four varieties of prickly pear from three localities in the municipality of Salinas, San Luis Potosí.

Variety	Locality	Moisture (%)	Ashes (%)	TSS (°Brix)	pH	TA (% citric acid)	TSS (g 100 g^{-1} FW)
Cardona	Salinas de Hidalgo	$85.48^{ab}\pm0.28$	$0.28^{a}\pm0.01$	$14.34^{a}\pm0.19$	$6.15^{a}\pm0.06$	$0.02^{b}\pm0.01$	$17.13^{b}\pm0.63$
	La Reforma	$86.50^{a}\pm1.03$	$0.34^{a}\pm0.02$	$14.00^{a}\pm0.29$	$6.11^{a}\pm0.05$	$0.03^{a}\pm0.01$	$16.68^{b}\pm0.65$
	Diego Martín	$84.58^{b}\pm0.29$	$0.32^{a}\pm0.02$	$14.36^{a}\pm0.28$	$6.16^{a}\pm0.09$	$0.03^{a}\pm0.01$	$19.47^{a}\pm0.58$
Naranjona	Salinas de Hidalgo	$85.73^{a}\pm0.36$	$0.40^{a}\pm0.03$	$14.04^{b}\pm0.19$	$6.59^{b}\pm0.02$	$0.02^{a}\pm0.01$	$11.46^{c}\pm0.47$
	La Reforma	$83.50^{a}\pm0.75$	$0.39^{a}\pm0.02$	$15.84^{a}\pm0.34$	$6.87^{a}\pm0.03$	$0.03^{a}\pm0.01$	$18.51^{a}\pm0.75$
	Diego Martín	$85.26^{a}\pm0.56$	$0.35^{a}\pm0.02$	$12.73^{b}\pm0.74$	$6.88^{a}\pm0.05$	$0.01^{b}\pm0.01$	$14.65^{b}\pm0.40$
Cristalina	Salinas de Hidalgo	$87.34^{a}\pm1.09$	$0.18^{b}\pm0.01$	$12.76^{a}\pm0.23$	$6.43^{a}\pm0.03$	$0.02^{a}\pm0.01$	$19.57^{ab}\pm0.89$
	La Reforma	$85.18^{a}\pm0.53$	$0.22^{a}\pm0.01$	$11.60^{b}\pm0.53$	$6.27^{b}\pm0.01$	$0.02^{a}\pm0.01$	$16.52^{b}\pm0.83$
	Diego Martín	$87.08^{a}\pm1.06$	$0.26^{a}\pm0.02$	$12.60^{a}\pm0.37$	$6.16^{b}\pm0.02$	$0.01^{b}\pm0.01$	$22.99^{a}\pm1.70$
Fayayuca	Salinas de Hidalgo	$85.51^{a}\pm0.37$	$0.01^{a}\pm0.24$	$14.87^{a}\pm0.31$	$6.42^{b}\pm0.02$	$0.03^{a}\pm0.01$	$17.19^{c}\pm0.50$
	La Reforma	$85.07^{a}\pm0.38$	$0.02^{a}\pm0.23$	$14.33^{a}\pm0.21$	$6.54^{b}\pm0.05$	$0.02^{b}\pm0.01$	$25.84^{a}\pm0.93$
	Diego Martín	$84.97^{a}\pm1.44$	$0.02^{a}\pm0.23$	$14.09^{a}\pm0.38$	$6.86^{a}\pm0.03$	$0.02^{b}\pm0.01$	$20.06^{b}\pm0.47$

TSS=total soluble solids; pH=potential hydrogen; TA=titratable acidity; TSS=total soluble sugars; ±=accuracy of an approximation; S.E.=standard error. Means that do not share a letter are significantly different according to Tukey (α=0.05). The variety between locations was the point of comparison.

(4.05%), and other fruits (Rubio-Ochoa *et al.*, 2019; Villarreal-Fuentes *et al.*, 2019). The low percentage of acidity in Naranjona and Cristalina varieties (0.01% to 0.03% citric acid) influences consumer preference; therefore, acidity is an important factor to determine the most suitable processing conditions for any fruit (Sáenz, 2000).

Total soluble sugars (TSS). The green to white crystalline fruits showed a high TSS concentration, particularly the Cristalina variety (22.99 g 100 g^{-1} FW) of Diego Martín and the Fafayuca variety (25.84 g 100 g^{-1} FW) of La Reforma. The TSS concentration in the four varieties varied significantly between locations, showing remarkably higher contents in La Reforma and Diego Martin. The difference in sugar concentration may be caused by the level of fruit ripening (Zenteno-Ramírez *et al.*, 2015). The low amount of total soluble sugars in the pulp of *Opuntia* fruits is due to the high temperatures (>30 °C) to which they are exposed, resulting in the reduction of their third phenological stage, corresponding to the development of the fruit (Inglese *et al.*, 2017; Nassrallah *et al.*, 2021).

These results are possibly the consequence of less competition for water, soil, and light resources in the backyard orchards than in commercial orchards, which have a higher density of plants per unit area. Climatic factors (temperature and precipitation) possibly had a lesser influence on these characteristics, given the closeness between locations.

CONCLUSIONS

The fruits of four varieties destined for commercialization (Salinas de Hidalgo) had the following physical characteristics: lower total fresh weight and smaller size.

The results of the four varieties of *Opuntia* fruits grown in the municipality of Salinas, San Luis Potosí, have variable physicochemical characteristics. Therefore, the harvesting practices adopted by farmers do not seem to be uniform, since the location of the fruit in the cladode of the prickly pear cactus is important for its harvesting.

Overall, the Cristalina and Fafayuca varieties of the *Opuntia albicarpa* species had larger fruits (length, diameter, and weight), as well as a high content of total soluble solids and total soluble sugars. These characteristics could contribute to consumer preference.

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REFERENCES

Abbas, E. Y., Ezzat, M. I., El Hefnawy, H. M. & Abdel Sattar, E. (2022). An overview and update on the chemical compolocalidadn and potential health benefits of *Opuntia ficus-indica* (L.) Miller. *Journal of Food Biochemistry*, doi:10.1111/jfbc.14310.

Cabrera, S. (2021). Procesado de tuna (*Opuntia ficus-indica*) como propuesta de aprovechamiento de frutos no comercializables producidos en San Sebastián Villanueva, Puebla. Tesis de licenciatura, Benemérita Universidad Autónoma de Puebla.

Corrales-García, J. (2011). Perspectivas agroindustriales de la postcosecha de nopalito y la tuna. IX Simposium-Taller Nacional y II Internacional sobre "Producción y Aprovechamiento del Nopal y Maguey", 12-13.

Díaz, E.M., Rodríguez, E.M. & Díaz, C. (2007). Chemical characterization of *Opuntia dillenii* and *Opuntia ficus-indica* fruits, *Food Chemistry, 103*(1): 38-45.doi: 10.1016/j.foodchem.2006.06.064.

Feugang, J.M., Konarski, P., Zou, D., Stintzing, F.C. & Zou, C. (2006). Nutritional and Medicinal Use of Cactus Pear (*Opuntia* spp.) Cladodes and Fruits. *Front. Biosciencia*, (11): 2574–2589.

Inglese, P., Mondragon, C., Nefzaoui, A., Saenz, C., Taguchi, M., Makkar, H. & Louhaichi, M. (2017). Crop ecology cultivation and uses of cactus pear. The Food and Agriculture Organization of the United Nations and the International Center for Agricultural Research in the Dry Area, Rome, Italy.

Lamia, I., Zouhir, C. & Youcef, A. (2018). Characterization and transformation of the *Opuntia ficus indica* fruits. *Journal of Food Measurement and Characterization, 12*(4): 2349-2357. doi:10.1007/s11694-018-9851-z.

Martins, M., Ribeiro, M. H. & Almeida, C. M. (2023a). Physicochemical, nutritional, and medicinal properties of *Opuntia ficus-indica* (L.) Mill. and its main agro-industrial use: A Review. *Plants, 12*(7): 1512. doi: 10.3390/plants12071512.

Martins, M., Ribeiro, M. H., Miranda, A., Lopes, S., Franco, R., Paiva, J. & Almeida, C. M. (2023b). New foods with history: nutritional and toxic profile of prickly pear. *Journal of Food Measurement and Characterization, 17*(1): 956-972. doi:10.1007/s11694-022-01680-z.

Montreuil J, Spik G, Fournet B, Toillier T. (1997). Nonenzymatic determinations of carbohydrates. In: Analysis of Food Contituents. JL Multon (ed). Wiley-VCH. Inc. New York. USA. Pp. 112-114.

Nassrallah, A. A., Khodaeiaminjan, M. & Kamal, K. (2021). Profile and biological properties of the main phenolic compounds in cactus pear (*Opuntia* spp.). *Opuntia* spp. Chemistry, Bioactivity and Industrial Applications, In: Ramadan M.F, Ayoub, T.E.M., & Rohn, S. (Eds.). pp 345-354.

Patil, K. V., & Dagadkhair, A. C. (2019). Physicochemical characteristics and antioxidant potential of *Opuntia* fruit: A review. *The Pharma Innovation Journal, 8*(6): 376-380.

Paucara, C. M. y Del Castillo, C.R. (2021). Caracterización física y química de la tuna (*Opuntia ficus indica*) en el municipio de Luribay, La Paz – Bolivia. *Revista Estudiantil AGRO-VET Facultad de Agronomía, Universidad Mayor de San Andrés, La Paz, Bolivia 5*(1): 5-12. Disponible en: https://agrovet.umsa.bo/index.php/AGV/article/view/45.

Reyes-Agüero, J. A., Aguirre, J. R., Carlín, F. y González-Durán, A. (2009). Catálogo de las principales variedades silvestres y cultivadas de *Opuntia* en la Altiplanicie Meridional de México. UASLP., SASGARPA y CONACYT San Luis Potosí, S.L.P. México.

Rubio-Ochoa, E., Pérez-Sánchez, R., Ávila-Val, T. C., Gómez- Leyva, J. F. & García- Saucedo, P. A. (2019). Propiedades fisicoquímicas de frutos silvestres de *Rubus* con potencial nutracéutico y alimenticio. *Revista Mexicana de Ciencias Agrícolas, 10*(SPE23): 291-301.

Sáenz, C. (2000). Processing technologies: an alternative for cactus pear (*Opuntia* spp.) fruits and cladodes. *Journal of Arid Environments, 46*(3): 209-225.

Sandoval-Trujillo, S.J., Ramírez-Cortés, V. & Hernández-Bonilla, B.E. (2019). Alternativas de producción del nopal en el Estado de México. *Vínculatégica, 5*(2): 1349-1359.

Silva, M. A., Albuquerque, T. G., Pereira, P., Ramalho, R., Vicente, F., Oliveira, M. B. P. & Costa, H. S. (2021). *Opuntia ficus-indica* (L.) Mill.: A multi-benefit potential to be exploited. *Molecules*, (26): 951. doi:10.3390/molecules26040951.

Villarreal-Fuentes, J. M., Alia-Tejacal, I., Pérez-Pérez, X. D., Espinosa-Zaragoza, S., Marroquin-Agreda, F. J. y Núñez-Colín, C. A. (2019). Caracterización fisicoquímica de frutos de ciruela mexicana (*Spondias purpurea* L.) en el Soconusco, Chiapas. *Ecosistemas y recursos agropecuarios, 6*(17): 219-229.

Zenteno-Ramírez, G., Juárez-Flores, B. I., Aguirre-Rivera, J. R., Ortiz-Pérez, M. D., Zamora-Pedraza, C., y Rendón-Huerta, J. A. (2015). Evaluación de azúcares y fibra soluble en el jugo de variantes de tunas (*Opuntia* spp.). *Agrociencia, 49*(2): 141-152.

Zenteno-Ramírez, G. (2016). Evaluación de la pulpa de variantes de tuna como alimento funcional. Tesis Doctoral. Universidad Autónoma de San Luis Potosí, Programas Multidisciplinarios de Posgrado en Ciencias Ambientales, pp 134. Repositorio nacional CONACYT.

Colegio de Postgraduados

Prevalence of productive impact diseases in cattle in Gomez Farias County, Jalisco, Mexico

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ABSTRACT

Objective: To estimate the incidence of productive impact diseases in San Andrés Ixtlán, Gómez Farías county, Jalisco, México; through the monthly collection from clinical cases data, attended from January 2019 to January 2020, in seven smallholders dairy farms.

Design/Methodology/Approach: Seven different smallholder livestock production units, mainly semi-stalled dairy cows, were monitored from January 2019 to January 2020, To calculate the frequency of the disease, the prevalence rate (PR) and the cumulative incidence rate (CIR) were determined, through the following equations: PR=(Total cases in a population at a given place and time / Total population at that place and given time)$\times 10^n$ and CIR=(Number of new cases in period and place / Total population at the beginning of the period in that place)$\times 10^n$

Results: According to 184 clinical cases, the metabolic diseases (21.20%) showed the highest PR, followed by parasitic (14.67%), bacterial (13.04%) and trauma (7.07%). For the CIR, diseases caused by gastrointestinal parasites (12%), showed the highest incidence, followed by mastitis (6%) and in third place malnutrition (5%). Gastrointestinal parasites of the genus *Moniezia* spp. (27.27%) and *Trichuris* spp. (4.55%) were found with higher and lower prevalence, respectively.

Study Limitations/Implications: The lack of information from regional studies that describe the productive impact diseases faced by smallholders dairy farm in the different regions of the state of Jalisco.

Findings/Conclusions: It is concluded that the most frequently problems are metabolic, followed by infestations by gastrointestinal parasites, especially those of the genus *Moniezia* spp.

Keywords: Cattle, metabolic problems, parasites.

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INTRODUCTION

In Mexico, produced 2,081,261 million tons of beef meat (SIAP, 2022) and 12,563,699 million litter of milk, with a dairy cow inventory of 2.3 million of heads in 2020 (SIAP, 2022). At the national level, Mexico has a cattle inventory of 35,224,960 heads, the country is recognized for being part of the top 10 of countries that produce cattle in the world, where Veracruz has the first place in cattle production with 4,386,162 heads and in second place the state of Jalisco with 3,326,573 of cattle heads (SIAP, 2022).

Therefore, the livestock sector must be aware of the different diseases that could be a challenge for its production. Metabolic disorders are a group of diseases, generally due to poor nutritional management that can affect cattle at any productive stage, such as ketosis, hypocalcemia and downer cow syndrome, which affect dairy cows immediately after calving. These disturbances in the metabolism of cows cause serious economic losses in terms of strong reduction in milk production and reproductive problems. It is estimated that the effects of these diseases are of great importance to evaluate the costs-benefits of diagnosis, treatment, and preventive medicine (Senthilkumar *et al.*, 2013). There is also a relationship between the incidence of metabolic diseases and nutritional disorders such as displaced abomasum, hypocalcemia and hypomagnesemia, ketosis and others such as mastitis; and reproductive problems such as placental retention and metritis, (Correa & Carulla, 2009), in some cases disorders such as tympany and rumen acidosis can be caused by ignorance in the handling of feed ingredients such as soymeal and ground corn, respectively, by producers mainly in small semi-stalled farms (Alfonso-Ávila *et al.*, 2012).

Also, gastrointestinal parasites, such as *Haemonchus* spp., *Cooperia* spp., *Trichostrongylus* spp., *Oesophagostomum* spp. and *Ostertagia* spp., cause estimated economic losses for the Mexican livestock industry calculated at US $ 445,096,562, while the losses due to *Eimeria* spp., amount to US $ 23,781,491 (Rodríguez-Vivas *et al.*, 2017), so the diseases caused by these parasites represent a great challenge for producers.

In the same way, diseases that are caused by virus, like the case of infectious bovine rhinotracheitis (IBR), which is associated with retained placenta (Montiel-Olguín *et al.*, 2019) and the bovine viral diarrhea virus (BVDV), bacterial infections such as brucellosis, leptospirosis and infections by *Staphylococcus* spp. and coliforms, which also have an incidence and distribution in livestock herds in Mexico, with a great impact on the productive and reproductive indicators, and also economic by the livestock herds holders (Castañeda-Vázquez *et al.*, 2013; Gutiérrez-Hernández *et al.*, 2020).

Therefore, the aim of this study was to estimate the incidence of productive impact diseases and the prevalence rate of gastrointestinal parasites in semi-stalled dairy cows in San Andrés Ixtlán, Gómez Farías Municipality, Jalisco; by collecting data from clinical cases attended from January 2019 to January 2020.

MATERIALS AND METHODS

Location and production data of the herds of the study site

This study was carried out in San Andrés Ixtlán, Gómez Farías county, Jalisco, Mexico. This place is the second most populous in the county, representing 35.1% of the total population of the county (16,431 habitants). It has a local livestock association; however, it does not have an updated record of the livestock heads or livestock holders in the county, neither by locality nor by county, but, according to SIAP (SIAP 2022), the county reported an annual production of 932.79 tons of live cattle (IIEG, 2022).

San Andrés Ixtlán is located at 1551 meters above sea level, with a semi-warm and semi-humid weather, the average annual temperature is 16.1 °C, while its average maximum and minimum oscillate between 27.2 °C and 4.1 °C, respectively. The average annual rainfall is 1,174 mm. The dominant land use in the county is forest occupying 55.4%.

(IIEG, 2022). Seven different smallholder livestock production units, mainly semi-stalled dairy cows, were monitored, and data from 184 clinical cases were collected from January 2019 to January 2020, with the intention of estimating the incidence and prevalence of diseases present in the region.

Diagnostic tests

In cows that presented constant weight loss, diarrheal syndrome, shaggy hair, pale gums (anemia) and sunken eyes, in addition to taking their clinical history, feces samples were collected from each animal and a coproparasitoscopic test was performed in the Microbiology Laboratory of the Centro Universitario del Sur of the University of Guadalajara, the flotation method with sucrose solution as described by Sixtos (2011), were conducted to make a diagnosis.

For the parasite identification, the table of gastrointestinal parasites in cattle and sheep from the Bayer Laboratory was used, as well as the identification table of coprological research of domestic animals from the Chinoin Laboratory (Mexico), particularly in cattle.

Study design

To calculate the frequency of the disease (FD), the prevalence rate (PR) and the cumulative incidence rate (CIR) were determined, through the following equations described by Martínez (2010):

PR=(Total cases in a population at a given place and time / Total population at that place and given time)$\times 10^n$

CIR=(Number of new cases in period and place / Total population at the beginning of the period in that place)$\times 10^n$

RESULTS AND DISCUSSION

During the period covered by this study, a total of 184 cattle were diagnosed, only 106 of them presented clinical problems attributed to various etiological agents, representing a population prevalence of 57.61% (Table 1).

Among the diagnosed cases, those due to metabolic problems are the most recurrent throughout the year, followed by parasitic, bacterial, and finally trauma such as blows, bites, lacerations, etc., mainly due to poor facilities of the production units and the presence of

Table 1. Prevalence rate according to the type of disease

Disease	Number of cases	Prevalence rate %
Metabolic	39	21.20
Parasitism	27	14.67
Bacterial	24	13.04
Traumatism	13	7.07
Undetermined	3	1.63

feral dogs. Figure 1 showed an average of approximately 9 cases per month, with January and September being the months with the highest number of disease presence, followed by the months of May and July, stabilizing the cases from October and with a lower number of cases in January 2020.

In this study, the increase in diseases in the months of January and September can be associated with the fact that bacterial diseases are more recurrent during winter (January) and in the rainy seasons (May-October), since during these months the temperature changes , humidity and rangelands conditions, together with the winds, cause cold stress, affecting the immune system and with it the presence of opportunistic agents that cause damage to the animal's organism (Peter *et al.*, 2015).

On the other hand, metabolic diseases are frequent throughout the year, since they are the result of poor management in cattle herds (García & Vázquez, 2020), this is due to the fact that most of the time the livestock grazes in overgrazed and poorly managed rangelands, double-purpose or semi-stalled farms in Mexico, and especially in the southern region of Jalisco, are characterized by poor nutritional management, poor mineral supplementation and only attempts to improve nutrition through corn and sugar cane stubbles, concentrates and rarely with the use of corn silages, which leads to a low productive yields and low reproductive efficiency (Magaña-Monforte *et al.*, 2006). Therefore, malnutrition appeared in third place among the problems detected with a 5% CIR (cumulative incidence rate), only after gastrointestinal parasites and mastitis, with a CIR of 12 and 6%, respectively (Table 2).

Since most of the local livestock smallholders focus on milk production, mastitis is a common problem in this type of production units, the lack of an adequate milking routine by mostly of the owners since these units are managed under a cow-calf system and manually milking. In a study carried out by Castañeda-Vázquez *et al.* (2013) in 33 dairy herds from different regions of the state of Jalisco from 67 to 54.5% of the cows presented subclinical and clinical mastitis, respectively. The foregoing gives us an epidemiological vision that in the state the main pathogens that cause mastitis are ***Staphylococcus aureus***, ***S. agalactiae***,

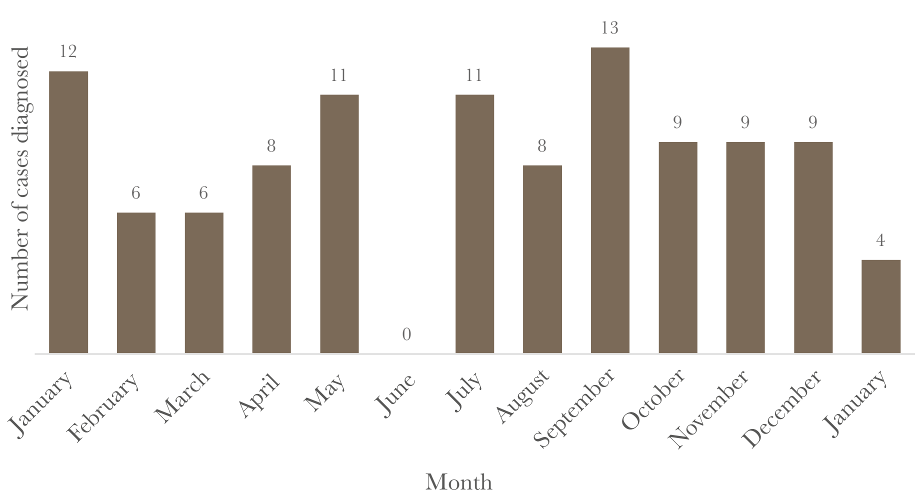

Figure 1. Number of cases diagnosed per month (2019-2020).

Table 2. Cumulative incidence rate (CIR) of detected diseases (n=184).

Disease	CIR %
Gastrointestinal parasites	12
Mastitis	6
Animal malnutrition	5
Tympanism	4
Rumen acidosis	4
Traumatism [a]	4
Retained placenta	3
Pyometra	3
Respiratory tract infection	2
Tick infestation	2
Mechanical diarrhea	2
Poisoning by toxic plants	2
Pododermatitis	2
Nipple laceration	2
Abortion	2
Anaplasmosis y piroplasmosis	1
Omphalophebitis	1
Actinomycosis	1
Ulcer in mammary gland	1

[a] Traumatism was considered those caused by a sharp object to the legs or hooves and dog bites.

Corynebacterium spp. and coliforms, in that order, so future studies in the southern region of Jalisco should consider the identification of pathogens, to contrast these findings at the state level, to adapt milking routines for the dairy cow producers in the region.

In the case of gastrointestinal parasites Fernández *et al.* (2015), observed that the highest number of parasites in cattle grazed in the months of May and June, probably because after the dry season, the parasites have not yet reached their full development.

Table 2 shows the CIR of the diseases presented during the year of the present study where it can be found that infestations by gastrointestinal parasites are those that showed a higher incidence in a year. Helminth infestations are one of the most important causes of diseases and low productivity in cattle in the world, being even more notorious in temperate and tropical climates since these conditions support the incidence and presence of gastrointestinal parasites in cattle, causing not only clinical parasite problems, but also effects in the subclinical phase that are not visible and that cause deficiencies in the use of nutrients, which increases losses in animal production (Vercruysse & Claerebout, 2001; Molento *et al.*, 2011), the above in according with the findings in this study, where gastrointestinal parasites (12%) represented the highest CIR and could be associated with some metabolic problems (21.20%) that occupy the highest prevalence rate in this studio (Table 1).

Rodríguez-Vivas *et al*. (2001), conducted a review of 3,827 bovine samples taken between 1984 and 1999, where they found that the most frequent gastrointestinal parasites were from the *Coccidia* family (71.57%) and *Strongylida* (60.64%), on the other hand, the most common genera were *Strongyloides* spp. (9.87), *Trichuris* spp. (8.28), and *Moniezia* spp. (3.86) these results differ from those found in this study, where gastrointestinal parasites of the genus *Moniezia* spp. (27.27%) were found with the highest prevalence, while the genus *Trichuris* spp. (4.55%) was the one with the lowest rate in this study (Table 3).

This contrasts with the results obtained in a study carried out by Munguía-Xóchihua *et al*. (2019), where the most abundant parasites of the nematode group were *Haemonchus* spp. (79.5%), *Oesophagostomum* spp. (40.4%) and *Trichostrongylus* spp (34.8%) in southern Sonora and with those reported (Fernández *et al*., 2015), in Hidalgotitlan, Veracruz where they found that the *Cooperia* spp. nematode occupied 49% of the total infestation; followed by *Ostertagia* spp. (15%), *Haemonchus* spp. (15%), *Trychostrongylus* spp. (7%), *Moniezia* spp. (5%), *Toxocora vitolorum* 4%, *Trichruris ovis* (4%) and *Chavertia ovina* (1%). This may be due to the agroecological conditions of the places where these studies were carried out. In the case of monieziosis, occurs in grazing cattle, this is common in the semi-stalled systems of Gomez Farias, where infected cattle contaminate the grazing areas with eggs of this cestode and where the intermediate hosts (oribatid mites) help to maintain the life cycle of the parasite (Quiroz, 2011). The domestic animals and particularly cattle are parasitized by a large variety of nematodes, cestodes and protozoa, which makes it necessary to carry out more local studies of this type to identify and quantify the economic impact of parasitic infections in cattle in the southern region of Jalisco, Mexico, and with this to be able to establish control programs parasite control adapted to each region of the country.

The high incidence of parasites in this study not only represents a health problem for cattle, but also an economic one for producers, since in countries with more rigorous management practices and health programs such as the United States, it has been calculated that the average losses costs for parasites for the livestock industry was US $50.67 animal/year, which represents 17.94% of the total losses within a herd (Rashid *et al*., 2019). In Brazil, it was estimated that at least US$13.96 million were lost annually due to the presence of internal and external parasites in cattle (Grisi *et al*., 2014). While, in Mexico considering the national bovine inventory registered in 2013 that had 32.40 million heads,

Table 3. Prevalence rate of gastrointestinal parasites*

Genera	Prevalence rate (%)
Moniezia spp.	27.27
Haemonchus spp.	18.18
Ostertagia spp.	13.64
Cooperia spp.	9.10
Chabertia spp.	9.10
Eimeria spp.	9.10
Trichostrongylus spp.	9.10
Trichuris spp.	4.55

*Determination by flotation method.

gastrointestinal parasites represented an annual loss per head of US $43.57 (Rodríguez-Vivas *et al*., 2017). For the regional and even national environment, the number of studies related to monitoring the economic impact of gastrointestinal parasites is very limited. In the present work, a monitoring investigation was carried out in order to detect the incidence of productive diseases in semi-stalled dairy cows. The general panorama obtained in the present study demonstrates the magnitude and importance of the challenges faced by milk producers that are managed under semi-stall systems in Mexico, so future studies that take into account the economic impact are necessary to generate useful information to maximize the profitability of the livestock industry together with the integration of sustainable and comprehensive management strategies (Cerdas, 2013; Olivares-Pérez *et al*., 2015; Rodríguez-Vivas *et al*., 2017).

CONCLUSION

In this study, it was identified that the most frequently problems are metabolic, followed by gastrointestinal parasites, especially *Moniezia* spp. In addition, the herds in this study located in San Andrés Ixtlán, Gómez Farías county, Jalisco, Mexico, showed a moderate to low probability of presenting bacterial diseases such as mastitis and actinomycosis, respectively.

It is concluded that the owners of dairy cows in semi-stalled systems in the town of San Andres Ixtlan face problems of productive impact generally associated with deficient health and nutritional management programs, for which it's recommended to implement prevention and control of these diseases, as well as adequate nutritional supplementation according to the productive stage of cattle to reduce economic losses in animal production, likewise the need for regional studies to determine the problems faced by small dairy farmers in the different regions of the state of Jalisco.

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REFERENCES

Alfonso-Ávila, Á. R., Wattiaux M. A., Espinoza-Ortega, A., Sánchez-Vera, E., & Arriaga-Jordán, C. M. (2012). Local feeding strategies and milk composition in small-scale dairy production systems during the rainy season in the highlands of Mexico. *Tropical Animal Health and Production. 44*(3):637–644. Doi: 10.1007/s11250-011-9947-5

Castañeda-Vázquez, H., Jäger, S, Wolter, W., Zschöck, M., Castañeda-Vazquez, M., & El-Sayed, A. (2013). Isolation and identification of main mastitis pathogens in Mexico. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia. 33*(2):377–382. Doi: 10.1590/S0102-09352013000200012

Cerdas-Ramírez, R. (2013). Formulación de raciones para carne y leche. Desarrollo de un módulo práctico para técnicos y estudiantes de ganadería de Guanacaste, Costa Rica. *InterSedes. 14*(29): 126–153. http://www.scielo.sa.cr/scielo.php?script=sci_arttext&pid=S221524582013000300009&lng=en&nrm=iso

Correa, H., & Carulla, J. (2009). Relación entre nutrición y salud en hatos lecheros. *Revista de La Facultad de Medicina Veterinaria y de Zootecnia. 56*(3):273–291. https://revistas.unal.edu.co/index.php/remevez/article/view/13933

Fernández, A., Román, A., Juárez, E., Romero, D., Romero, M., & Felipe, I. (2015). Prevalencia de nematodos gastroentéricos en bovinos doble propósito en 10 ranchos de Hidalgotitlan Veracruz, México. *Abanico Veterinario. 5*(2):13-18.

García, D., & Vázquez, J. (2020). Prevalencia de parásitos gastrointestinales en bovinos hembras adultas de los cantones occidentales de la provincia del Azuay. *Centrosur. 1*(5):11–17. Doi: 10.37959/cs.v1i5.23

Grisi, L., Leite, R. C., Martins, J. R., Barros, A. T., Andreotti, R., Cançado P. H., León, A. A., Pereira, J. B., & Villela, H. S,. (2014). Reassessment of the potential economic impact of cattle parasites in Brazil. *Brazilian Journal Veterinary Parasitology. 23*(2):150–156. Doi: 10.1590/S1984-29612014042

Gutiérrez-Hernández, J., Palomares-Resendiz, G., Hernández-Badillo, E., Leyva-Corona J., Díaz-Aparicio, E., & Herrera-López, E. (2020). Frecuencia de enfermedades de impacto reproductivo en bovinos de doble propósito ubicados en Oaxaca, México. *Abanico Veterinario*. 10.1–11. ISSN: 2448-6132. Doi: 10.21929/abavet2020.22

IIEG. (2022). Instituto de Información Estadística y Geográfica. Gómez Farías: Diagnóstico del municipio. Recuperado: https://iieg.gob.mx/ns/wp-content/uploads/2022/11/Gom%C3%A9z-Far%C3%ADas.pdf

Magaña-Monforte, J. G., Ríos-Arjona G., & Martínez-González, J. C. (2006). Los sistemas de doble propósito y los desafíos en los climas tropicales de México. *Archivos Latinoamericanos de Producción Animal. 14*(3):105–114. https://www.bioline.org.br/pdf?la06019

Martínez, J. J. (2010). El proceso epidémico. In: C. J. Jaramillo y J. J. Martínez (Eds.), Epidemiología Veterinaria. Pp. 33–52. Manual Moderno. México

Molento, M. B., Fortes, F. S., Pondelek, D. A. S., Borges, F.A., Chagas, A. C. S, Torres-Acosta, J. F. J., & Geldhof, P. (2011). Challenges of nematode control in ruminants: Focus on Latin America. *Veterinary Parasitology. 180*(1–2):126–132. Doi: 10.1016/j.vetpar.2011.05.033

Montiel-Olguín, L. J., Estrada-Cortés, E., Espinosa-Martínez, M. A., Mellado, M., Hernández-Vélez, J. O., Martínez-Trejo, G, Hérnández-Andrade, L., Hérnández-Ortíz, R., Alvarado-Islas, A., Ruiz-López, F. J., & Vera-Avila, H. R. (2019). Factores de riesgo a nivel de establo asociados con el desempeño reproductivo en el sistema de producción de leche a pequeña escala en México. *Revista Mexicana de Ciencias Pecuarias. 10*(3):676–691. Doi: 10.22319/rmcp.v10i3.4825

Munguía-Xóchihua, J., Leal-Franco, I., Muñoz-Cabrera, J., Medina-Chu, M., Reyna-Granados, J., & López-Castro, P. (2019). Frecuencia de parásitos gastrointestinales en bovinos del sur de Sonora, México. *Abanico Veterinario*. 9:1–11. Doi: 10.21929/abavet2019.919

Olivares-Pérez, J, Kholif, A. E., Rojas-Hernández, S., Elghandour, M. M. M. Y., Salem, A. Z. M., Bastida, A. Z., Velázquez-Reynoso, D., Cipriano-Salazar, M., Camacho-Díaz, L. M., Alonso-Fresán, M. U., & DiLorenzo, N. (2015). Prevalence of bovine subclinical mastitis, its etiology and diagnosis of antibiotic resistance of dairy farms in four municipalities of a tropical region of Mexico. *Tropical Animal Health and Production. 47*(8):1497–1504. Doi: 10.1007/s11250-015-0890-8

Peter, G. S., Gitau, G. K., Mulei, C. M., Vanleeuwen, J., Richards, S., Wichtel, J., Uehlinger, F., & Mainga, O. (2015). Prevalence of Cryptosporidia, Eimeria, Giardia, and Strongyloides in pre-weaned calves on smallholder dairy farms in Mukurwe-ini district, Kenya. *Veterinary World. 8*(9):1118–1125. Doi: 10.14202/vetworld.2015.1118-1125

Quiroz, H., Figueroa, J., Ibarra, F., & López, M. (2011). Epidemiología de enfermedades parasitarias en animales domésticos. México: UNAM. 655 p

Rashid, M., Rashid, M. I., Akbar, H., Ahmad, L., Hassan, M. A., Ashraf, K., Saeed, K., & Gharbi, M. (2019). A systematic review on modelling approaches for economic losses studies caused by parasites and their associated diseases in cattle. *Parasitology. 146*(2):129–141. Doi: 10.1017/S0031182018001282

Rodríguez-Vivas, R. I., Cob-Galera, L. A., & Domínguez-Alpizar, J. L. (2001). Frecuencia de parásitos gastrointestinales en animales domésticos diagnosticados en Yucatán, México. *Revista Biomédica. 12*(1):19–25. Doi: 10.32776/revbiomed.v12i1.253

Rodríguez-Vivas, R. I., Grisi, L., Pérez, A. A., Silva-Villela, H., Torres-Acosta, J. F. J., Fragoso-Sánchez, H., Romero-Salas, D., Rosario-Cruz, R., Saldiernah, F., & García-Carrasco, D. (2017). Potential economic impact assessment for cattle parasites in Mexico. *Review. Revista Mexicana De Ciencias Pecuarias. 8*(1):61–74. Doi: 10.22319/rmcp.v8i1.4305

Senthilkumar, V., Safiullah, A. M., Kathiravan, G., Subramanian, M., & Mani, K. (2013). Economic Analysis of Metabolic Diseases in Bovines: A Review. *International Journal of Advanced Veterinary Science and Technology. 2*(1):64–71. http://krishikosh.egranth.ac.in/handle/1/5810072837

SIAP. (2022). Servicio de Información Agroalimentaria y Pesquera. Recuperado: https://nube.siap.gob.mx/cierre_pecuario/

Sixtos C. Procedimientos y técnicas para la realización de estudios coproparasitoscópicos. *Virbac Al Día Animales de Compañía*. 24: 1-12. Recuperado: https://issuu.com/hitsoft/docs/arte_final_vad_ac_24

Vercruysse, J., & Claerebout, E. (2001). Treatment vs non-treatment of helminth infections in cattle: Defining the threshold. *Veterinary Parasitology. 98*(1–3):195–214. Doi: 10.1016/S0304-4017(01)00431-9

Colegio de Postgraduados

Productive response of two genotypes of chickens (Mexican Creole and Sasso), in confinement and grazing white clover (*Trifolium repens* L.)

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ABSTRACT

Objective: To determine the productive performance and forage consumption (*Trifolium repens* L.) of Mexican Creole and Sasso chickens, under two rearing systems (confinement or grazing).

Design/Methodology/Approach: One-hundred twenty-eight chickens (64 Mexican Creole (MC) and 64 Sasso (S)) of 35 d of age were randomly distributed in two production systems to obtain four repetitions (eight chickens per repetition) of each of the following genotype × system combinations: Mexican Creole in grazing, Mexican Creole in confinement, Sasso in grazing, and Sasso in confinement. A completely randomized experimental design with a 2×2 factorial arrangement was used, with genotype and production system as its main factors. The following variables were evaluated: feed consumption, weight gain, and feed conversion. Additionally, forage consumption in grazing birds was determined.

Results: The productive performance variables were not affected by the production system factors or by its interaction with the bird genotype. However, the genotype did influence the variables considered: the Sasso birds recorded better values ($p \leq 0.05$) than Mexican Creole specimens. Regarding forage consumption, no differences were observed ($p \leq 0.05$) between bird genotypes and, in both cases, the accumulated consumption at the 49 d of study was close to 60 g of DM.

Study Limitations/Implications: It is necessary to carry out a socioeconomic study as well as a defoliation level analysis with the aim of improving the use of the resource.

Findings/Conclusions: Mexican Creole birds had a lower productive performance with a similar forage consumption.

Keywords: Mexican Creole, Sasso, productive performance, forage consumption, *Trifolium repens* L.

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INTRODUCTION

In recent years, consumers have become increasingly interested in poultry products from non-intensive systems (Moyle *et al.*, 2014) that include slow-growing broilers (Sossidou *et al.*, 2011), such as the Mexican Creole and Sasso chickens. While the Sasso chicken is

generated by a global company specializing in poultry genetics (Sasso, 2022), the Mexican native chicken is a product of the natural selection of the birds brought by the Spanish conquistadors (Cuca-García, 2018). This genotype is found in rural communities, where they complement their diet with grains, insects, and even forage (Terrell, 2013). The last element is relevant for poultry meat production in alternative grazing systems (Lorenz *et al.*, 2013). Stadig *et al.* (2016) determined that confined Sasso chickens had a higher weight, but they did not find any differences in feed consumption and feed conversion between birds raised in grazing or confinement systems. Few studies have sought to determine the amount of forage consumed by chickens in grazing production systems. Mugnai *et al.* (2014) reported a consumption of 59 g of DM d^{-1}, while Dal Bosco *et al.* (2014) recorded a consumption range of 14 to 55 g of DM d^{-1} in naked neck chickens. The commercial exploitation and utilization of Mexico's poultry genetic resources requires evidence that supports their productive performance in face of the options available in the market for non-conventional production systems, such as Sasso chicken (Aman *et al.*, 2017). To date, no such information seems to exist in reference works.

MATERIALS AND METHODS

One hundred twenty-eight mixed chickens (64 Mexican Creole (MC) and 64 Sasso (S)) of 35 d of age were randomly distributed in two production systems (grazing or confinement), obtaining four repetitions (eight birds per repetition) of the following genotype × production system combinations: Mexican Creole in grazing, Mexican Creole in confinement, Sasso in grazing, and Sasso in confinement. While confined birds were kept in pens within a natural environment poultry house with movable side curtains, the birds in the grazing system had access to a white clover (*Trifolium repens* L.) meadow from 9:00 am to 5:00 pm and spent the rest of the day sheltered within the poultry house. A 5-cm layer of wood chip was laid inside 1.1×1.4 m pens (8 birds per pen, 5.2 birds m^2). Each pen had a 10 kg hopper feeder and a 5 L plastic poultry drinker. From 35 to 56 d of age, the chickens were fed a diet of 2,550 kcal of metabolizable energy per kg and 17% crude protein; subsequently and until they were 84 d old, they were offered commercial balanced feed (minimum CP, 17.5%; crude fat, 4%; minimum crude fiber, 4.5%; maximum ash, 5.5%; maximum humidity, 12%; NFE by difference, 55.5%). Food and water were offered *ad libitum*.

Productive performance

The following variables were recorded weekly with chickens from 35 to 84 d of age: feed consumption, weight gain, and feed conversion. Feed consumption was calculated according to the following equation:

$$\textit{weekly feed consumption}\ (\mathrm{g}) = \textit{food offered}\ (\mathrm{g}) - \textit{residual food}\ (\mathrm{g})$$

Weight gain was calculated as follows:

$$\textit{weekly weight gain}\ (\mathrm{g}) = \textit{weight at the end of the week}\ (\mathrm{g}) - \textit{weight at the beginning of the week}\ (\mathrm{g}).$$

Finally, feed conversion was calculated with the following formula:

$$\textit{feed conversion}\ (g)/(g) = \textit{feed consumption}\ (g)/\textit{weight gain}\ (g)$$

Additionally, variables were measured cumulatively for the study period (35 to 84 days of age).

Forage consumption determination

In each repetition, grazing MC and S birds had access to 42 m^2 (10.5×4 m) white clover meadows. Following the suggestions of Hasan *et al.* (2013) for slow-growing broilers, each bird had access to 5 m^2. In each repetition, the flock remained seven days within each plot. Subsequently, the group moved to an area that had been allowed to rest from grazing for 15 days. Each plot had a 5 L plastic poultry drinker and a 2 m^2 mobile shade. The consumption of white clover was determined using the methodology of Dal Bosco *et al.* (2014): a 0.75×0.75 m exclusion pen was set up in each repetition, to keep the chicken away from that area. In each 7 d period in which the meadow was occupied, a forage sample was taken randomly when the animals entered (GM_S, herbaceous mass present) and exited (GM_e, remaining forage after consumption), using a 0.75×0.75 m metal frame to measure the forage. In addition to the content in the exclusion pen (GM_u, undisturbed forage mass), the forage was cut 5 cm from the ground and subsequently placed in a paper bag to be weighed on a Metrology® BCH-5000 scale (Mexico) with a 5,000 g capacity and 0.5 g resolution. Once the weight of the forage sample had been obtained, the plant material was left to dry in the sun until a constant weight was reached, verified with the same scale. Finally, the GM_S, GM_e, and GM_u values were substituted in the equation proposed by Lantinga *et al.* (2004) to estimate forage consumption (FC):

$$FC = \left(GM_S - GM_e\right) + \left\{ \frac{\left[1 - \left(\frac{GM_e}{GM_S}\right)\right]}{-\ln\left[\frac{GM_e}{GM_S}\right]} \right\} \times \left(GM_u - GM_S\right)$$

Statistical analysis

The data of the studied variables were analyzed with the MIXED procedure of SAS version 9.3 (SAS, 2011), under a completely randomized experimental design with a 2×2 factorial arrangement, using genotype and production system as main factors. The effect of each factor was considered significant ($p \leq 0.05$). The adjusted means were compared using the Tukey's test.

RESULTS AND DISCUSSION

Overall, the genotype × production system interaction was not significant ($p > 0.05$) with respect to feed consumption, except from 56 to 70 d of age, when the S birds had a higher consumption in both systems than the MC, which reduced their consumption under confinement (Table 1). These results match the findings of Ponte *et al.* (2008), who reported that birds with access to grazing consume more balanced feed. Likewise, throughout the study period, S birds consumed 20-70% more feed ($p \leq 0.05$) (Table 1). The average consumption of S birds reached 101 g bird^{-1} d^{-1}; this figure was higher than the 40-87 g bird^{-1} d^{-1} range reported in previous studies with this same genotype under grazing conditions (Yitbarek *et al.*, 2016; Bayesa *et al.*, 2020). Meanwhile, the average MC consumption was 70 g bird^{-1} d^{-1}: there results are higher than those reported by Segura-Correa *et al.* (2004) and Matus-Aragón *et al.* (2021). These authors recorded those Mexican Creole chickens consumed between 52 and 63 g bird^{-1} d^{-1}. Finally, feed consumption was impacted ($p<0.05$) by the production system up to 70 d of age (except from 56 to 63 d); higher results were obtained in the confinement system than in grazing. These were not the expected results. Unlike the results obtained by Ponte *et al.* (2008), the consumption of concentrated feed should have increased as a consequence of the greater energy demanded by grazing. This increase would have been caused by the grains and oils that are the main source of energy in poultry diets (Terrell, 2013).

Table 2 shows no overall effect of the genotype × production system interaction or the production system itself ($p>0.05$) on weight gain. In contrast, S birds gained more weight than MC birds ($p<0.05$), recording 1,731 and 852 g, respectively, from 35 to 84 d of age. The weight gain (34 g bird^{-1} d^{-1}) matches the 13 and 34 g bird^{-1} d^{-1} reported for S chicken up to 56 d of age by Yitbarek *et al.* (2016) and Sanka *et al.* (2020). Nevertheless, the MC birds gained 852 g in weight during the 49 d of the study, a value lower than the

Table 1. Adjusted means (±SE) of feed consumption† (g) of Sasso and Mexican Creole chickens reared in grazing or confinement.

Factor	Level	Age (d)							
		35-42	43-49	50-56	56-63	63-70	70-77	77-84	35-84
Genotype	Sasso	556 a	665 a	694 a	592 a	756 a	850 a	852 a	4965 a
	Mexican Creole	456 b	534 b	498 b	400 b	515 b	476 b	491 b	3497 b
	standard error	16	7	19	18	12	40	33	128
System	Confinement	533 a	614 a	629 a	492	601 a	650	683	4179
	Grazing	479 b	584 b	562 b	500	670 b	675	659	4283
	standard error	16	7	19	18	12	40	33	128
Genotype × System	Sasso × Grazing	535	657	675	541 ab	756 a	860	849	4873
	Mexican Creole × Grazing	578	674	713	459 b	584 b	490	517	3693
	Sasso × Confinement	488	555	546	642 a	757 a	840	855	5057
	Mexican Creole × Confinement	423	512	449	342 c	446 c	461	464	3301
	standard error	22	10	27	541 ab	756 a	57	47	182
P value									
Genotype		*	***	***	***	***	***	***	***
System		*	*	*	NS	*	NS	NS	NS
Genotype × System		NS	NS	NS	*	*	NS	NS	NS

a,b,c: Mean values per column with a different letter are statistically different ($p \leq 0.05$). *** ($p \leq 0.0001$), * ($p \leq 0.05$), NS=not significant ($p>0.05$). † Feed consumption is relative to the consumption of balanced feed.

Table 2. Adjusted means (±SE) of weight gain (g) of Sasso and Mexican Creole chickens reared in pasture or confinement.

Factor	Level	Age (d)							
		35-42	43-49	50-56	56-63	63-70	70-77	77-84	35-84
Genotype	Sasso	251 a	210 a	268 a	236 a	252 a	227 a	287 a	1731 a
	Mexican Creole	115 b	127 b	134 b	114 b	114 b	109 b	132 b	852 b
	standard error	5	6	11	16	9	17	132	41
System	Confinement	181	170	193	192	171	152	235	1293
	Grazing	185	167	209	159	196	183	184	1290
	standard error	5	6	11	16	9	17	132	41
Genotype × System	Sasso × Grazing	255	205	284	198	286 a	266	269	1763
	Mexican Creole × Grazing	115	130	134	119	105 c	101	100	818
	Sasso × Confinement	246	216	253	275	217 b	187	305	1698
	Mexican Creole × Confinement	116	124	134	109	124 c	116	165	887
	standard error	8	8	16	23	13	23	34	58
P value									
Genotype		***	***	***	*	***	*	*	***
System		NS	NS	NS	NS	NS	NS	NS	NS
Genotype × System		NS	NS	NS	NS	*	NS	NS	NS

a,b,c: Mean values per column with a different letter are statistically different ($p \leq 0.05$). *** ($p \leq 0.0001$) * ($p \leq 0.05$), NS=not significant ($p>0.05$).

1,096 g reported by Matus-Aragón *et al.* (2021), while the daily weight gain at 49 d (17.4 g bird^{-1} d^{-1}) was close to the 14.5 g bird^{-1} d^{-1} reported by Segura-Correa *et al.* (2004).

In the case of feed conversion (Table 3), there were no differences ($p>0.05$) between the genotype × production system interaction and the production system as an individual factor. However, the genotype of the bird was significant ($p<0.05$): MC birds obtained a greater feed conversion than the S birds (1.0 to 1.6 g more food per each g of weight gain). S chickens had a conversion of 3.1 g g^{-1} at 49 d. This result is similar to the 2.8 to 2.9 g g^{-1} at 84 d of age reported by Rocha-Barros *et al.* (2009), but lower than the 2.7 to 3.4 g g^{-1} reported by Sanka *et al.* (2020). The conversion in Mexican Creole chickens was 4.2 g g^{-1} from 35 to 84 d of age. This value was higher than the 3.5-4.14 g g^{-1} range reported at 84 d by Matus-Aragón *et al.* (2021); however, at 49 d of age, the feed conversion reached 4.2 g g^{-1}, which is very similar to the 4.36 g g^{-1} found by Segura-Correa *et al.* (2004).

The productive performance of birds of zootechnical interest is determined by the genotype, age, environmental conditions, availability of nutrients, and the health status of the animals, among other factors (Lesson and Summers, 2001). The results obtained in the present research are related to the abovementioned factors, given the marked difference in weight gain and feed conversion between the genotypes of the birds under study. Sasso chickens recorded better values in both variables, as a result of the genetic selection process to which this commercial race has been subject to for many years (Aman *et al.*, 2017).

No differences in forage consumption were observed ($p \leq 0.05$) between the genotypes of the birds: in both cases, the animals consumed ≈60 g DM of forage in the 49 d of evaluation (Table 4). Few research have been carried out to quantify the forage consumption

Table 3. Adjusted means (±SE) of feed conversion [†] (g) of Sasso and Mexican Creole chickens reared in grazing or confinement.

Factor	Level	Age (d)							
		35-42	43-49	50-56	56-63	63-70	70-77	77-84	35-84
Genotype	Sasso	2.2 b	3.1 b	2.6 b	2.6 b	3.1 b	4.0	3.2	2.9
	Mexican Creole	3.8 a	4.2 a	3.8 a	3.6 a	4.7 a	4.0	4.6	4.2
	standard error	0.2	0.1	0.1	0.2	0.2	0.4	0.6	0.2
System	Confinement	3.3	3.8	3.5 a	2.9	3.6	4.5	3.1	3.4
	pasture	3	3.6	2.9 b	3.3	4.2	3.6	4.7	3.7
	standard error	0.2	0.1	0.1	0.2	0.2	0.4	0.6	0.2
Genotype × System	Sasso × Grazing	2.1	3.2	2.4	2.8	2.6 a	3.2	3.5	2.8
	Mexican Creole × Grazing	3.7	4	3.4	3.9	5.7 c	4.0	5.9	4.6
	Sasso × Confinement	2.3	3.1	2.8	2.4	3.6 b	4.8	2.9	3.0
	Mexican Creole × Confinement	4.2	4.5	4.2	3.3	3.6 c	4.1	3.2	3.7
	standard error	0.2	0.2	0.2	0.0	0.3	0.5	0.8	0.3
P value									
Genotype		***	***	***	*	*	NS	NS	NS
System		NS	NS	*	NS	NS	NS	NS	NS
Genotype × System		NS	NS	NS	NS	*	NS	NS	NS

a,b,c: Mean values per column with a different letter are statistically different ($p \leq 0.05$). SE=standard error, *** ($p \leq 0.0001$), * ($p \leq 0.05$), NS=not significant ($p > 0.05$). [†] Feed consumption is relative to the consumption of balanced feed.

of grazing birds; after a literature review, no studies were found about this variable for Sasso or Mexican Native chickens. However, when the present results are compared with the findings of Dal Bosco *et al.* (2014), naked neck chickens had a higher consumption (14 and 55 g DM bird^{-1} d^{-1}) than MC and S chickens (1.143 and 1.218 g DM bird^{-1} d^{-1}, respectively). Ponte *et al.* (2008) report that hybrid chickens (RedBro CouNu × RedBro M) consume 3.0 to 6.5 g of DM bird^{-1} d^{-1} of forage, while Rivera-Ferre *et al.* (2007) recorded an average consumption of 10.7 g of DM bird^{-1} d^{-1} among ISA-957 birds. The amount of forage consumed by birds of both genotypes in the present study was lower than the results of Dal Bosco *et al.* (2014) and Mirabito and Lubac (2001). Several factors limit forage consumption, including genotype, sex, age, and grazing schedule (Almeida *et al.*, 2012).

Table 4. Adjusted means (±SE) of forage consumption by Sasso and Mexican Creole chickens with access to grazing.

Level	Age (d)							
	35-42	43-49	50-56	56-63	63-70	70-77	77-84	35-84
Sasso	3.2	3.2	9.1	4.3	12.3	6.7	17.2	56.0
Mexican Creole	1.9	1.9	3.9	7.9	15.8	8.4	19.9	59.7
standard error	0.5	0.5	1.5	2.6	4.7	1.5	4.2	8.1
P value	NS	NS	NS	NS	NS	NS	NS	NS

a,b,c: Mean values per column with a different letter are statistically different ($p \leq 0.05$). *** ($p \leq 0.0001$), * ($p \leq 0.05$), NS=not significant ($p > 0.05$).

Furthermore, due to their digestive physiology, chickens prefer less fibrous ingredients (Martens *et al.*, 2012). Eyles (1963) reported that forage can represent up to 5% of the daily DM consumption of free grazing poultry. Likewise, not all types of birds make an optimal use of forage (Singh and Cowieson, 2013).

CONCLUSIONS

Regardless of their genotype, Mexican Creole and Sasso chickens tend to have similar productive performance, whether they are raised in confinement or grazing. Meanwhile, Sasso birds showed better weight gain and feed conversion values from 35 to 84 d of age than Mexican Creole chickens. Both genotypes of grazing chickens consume similar amounts of white clover: ≈60 g of DM in 49 days, *i.e.*, 1.143 and 1.218 g of DM bird^{-1} d^{-1}.

REFERENCES

Almeida GFD, Hinrichsen LK, Horsted K, Thamsborg SM, Hermansen JE. (2012). Feed intake and activity level of two broiler genotypes foraging different types of vegetation in the finishing period. *Poultry Science* 91, 2105–2113. doi:10.3382/ps.2012-02187

Aman, G., Bekele, B., Bereket, D., Gebriel, A., Tadesse, A., Edget, J. 2017. Production performance of Sasso (distributed by ethio-chicken private poultry farms) and Bovans brown chickens breed under village production system in three agro-ecologies of Southern Nations, Nationalities, and Peoples Regional State (SNNPR), Ethiopia. *International Journal of Livestock 8*(157). 145 – 157.

Bayesa, T., Ahmad, S., Wasihun, H., Mohamed, A. 2020. Effect of Age and Genetic Groups on Growth Performance of Crossed Koekoek and Sasso Chickens. *Biochemistry and Molecular Biology 5*(4). 44 – 49. Doi: 10.11648/j.bmb.20200504.11

Cuca-García, J. (2018). La avicultura de traspatio en México: Historia y caracterización. *Agro Productividad, 8*(4). 30–36. https://revista-agroproductividad.org/index.php/agroproductividad/article/view/669

Dal Bosco, A., Mugnai, C., Rosati, A., Paoletti, A., Caporali, S., Castellini, C. 2014. Effect of range enrichment on performance, behavior, and forage intake of free-range chickens, *Journal of Applied Poultry 23*(2). 137–145. Doi:10.3382/japr.2013-00814.

Eyles, D.E., 1963. Non-Ruminants. In: A.N. Worden, K.C. Sellers & D.E. Truibe (Eds), Animal Health, Production and Pasture. Longmans, London, pp. 359–383.

Hasan, E., Arda, Y., Nurel, D., Ahmet, S., Mustafa, D. 2013. Comparison of meat quality and fatty acid profile in slow-growing chicken genotypes feddiets supplemented with *Origanum vulgare* or *Melissa officinalis* leaves under the organic system. *British Poultry Science 56*(4). 477– 485. Doi: 10.4081/ijas.2013.e64

Lantinga, A., Neuteboom, J., Mei, J. 2004. Sward methods in Herbage Intake Handbook, 2nd ed. P.D. Penning, ed. The British Grassland Society, Reading, UK 2.23–52

Lesson, S. and Summers, J. 2001. Nutrition of the chicken, 4°edition. University of Guelph, Guelph, Ontario, Canada NIG ZW1, Department of animal & poultry science.

Lorenz, C., Kany, T. , Grashorn, M. 2013. Method to estimate feed intake from pasture in broilers and laying hens. *Archiv für Geflügelkunde. 77*(3). 160–165.

Martens S.T., Tassilo B., Jerome M., Lascano C. (2012). Alternative plant protein sources for pigs and chickens in the tropics - Nutritional value and constraints: A review. *Journal of Agriculture and Rural Development in the Tropics and Subtropics*. 113. 101-123. 2012.

Matus-Aragón, M., González-Cerón, F., Salinas-Ruiz, J., Sosa-Montes, E., Pro-Martínez, A., Hernández-Mendo, O., Cuca-García, J., Chan-Díaz, D. 2021. Productive performance of Mexican Creole chickens from hatching to 12 weeks of age fed diets with different concentrations of metabolizable energy and crude protein. *Animal bioscience 34*(11). 1794 – 1801 Doi: 10.5713/ab.20.0682

Mirabito, L., Lubac, L. 2001. Descriptive study of outdoor run occupation by "Red Label" type chickens. *British Poultry Science* 42. S16–S17.

Moyle, J.R., Arsi, K., Woo-Ming, A., Arambel, H., Fanatico, A., Blore, P.J., Clark, F.D., Donoghue, D.J., Donoghue, A.M. 2014. Growth performance of fast-growing broilers reared under different types of production systems with outdoor access: Implications for organic and alternative production systems, *Journal of Applied Poultry Research 23*(2). 212–220. Doi: 10.3382/japr.2013-00882.

Mugnai, C., Sossidou, E.N., Dal Bosco, A., Ruggeri, S., Mattioli, S., Castellini, C. 2014. The effects of husbandry system on the forage intake and egg nutritive characteristics of laying hens. *Journal of the Science of Food and Agriculture 94*(3). 459–467. Doi:10.1002/jsfa.6269.

Ponte, P., Rosado, C., Crespo, J., Crespo, D., Mourão, J., Chaveiro, M., Brás, J., Mendes, I., Gama, L., Prates, J., Ferreira, L., Fontes, C. 2008. Pasture Intake Improves the Performance and Meat Sensory Attributes of Free-Range Broilers, *Poultry Science 87*(1). 71–79 Doi:10.3382/ps.2007-00147

Rivera-Ferre, M.G., Lantinga, E.A., Kwakkel, R.P. 2007. Herbage intake and use of outdoor area by organic broilers: effects of vegetation type and shelter addition. *Journal or Animal Science 54*(3). 279–291 Doi:10.1016/S1573-5214(07)80020-0

Rocha-Barros, D.L., Sakomura, N.K., Cardoso, D., Dorigam, J.C., Marcato, S., Kochenborger, B. 2009. Crescimento e desempenho de linhagens de aves pescoço peladocriadas em sistema semi-confinado. *Ciências agrotecnologia, 33*(3). 875–881. Doi:10.1590/S1413-70542009000300030

Sanka, Y.D., Mbaga, S.H., Mutayoba, S.K. 2020. Evaluation of growth performance of Sasso and Kuroiler chickens fed three diets at varying levels of supplementation under semi-intensive system of production in Tanzania. *Tropical Animal Health and Production* 52. 3315 – 3322 Doi:10.1007/s11250-020-02363-x

SAS Institute Inc. 2011. SAS user's guide: statistics version. SAS Institute Inc., Cary, NC, USA. 959 pp.

Sasso, 2022. Guía de manejo para pollos de engorda. Hendrix Genetics.

Segura-Correa, J.C., Sarmiento-Franco, L., Magaña-Monforte, J.G., Santos-Ricalde, R. 2004 Productive performance of Creole chickens and their crosses raised under semi-intensive management conditions in Yucatan, Mexico, *British Poultry Science 45*(3). 342–345. DOI: 10.1080/00071660410001730833

Singh y Cowieson.2013.Range use and pasture consumption in free-range poultry production. *Animal Production Science 53*(11):1202-1208. DOI:10.1071/an13199

Sossidou, E., Dal Bosco, A., Elson, H., Fontes, C. 2011. Pasture-based systems for poultry production: Implications and perspectives. *World's Poultry Science Journal 67*(1). 47–58 Doi:10.1017/S0043933911000043.

Stadig, L.M., Rodenburg, T.B., Bert J.A., Barbara, D., Frank, A.M. 2016. Effects of free-range access on production parameters and meat quality, composition and taste in slow-growing broiler chickens. *Poultry Science 95*(12). 2971–2978. Doi:10.3382/ps/pew226.

Terrell, S. 2013. Pastured Poultry Nutrition and Forages. National sustainable agriculture information service. USA https://www.sare.org/wp-content/uploads/Pastured-Poultry-Nutrition-and-Forages.pdf (Recuperado: mayo 2022).

Yitbarek, M.B., Mersso, B.T., Wosen, A.M. 2016. Effect of dried blood-rumen content mixture (DBRMN) on feed intake, body weight gain, feed conversion ratio and mortality rate of SASSO C44 broiler chicks. *Journal of Livestock Science* 7. 139–149. DOI:10.13140/RG.2.1.4295.1286